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Original Article

Analysis of artifacts in oral and maxillofacial histopathologic sections and related reasons

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ABSTRACT

Background: Artifacts, artificial structures at microscopic section, may lead to incorrect diagnosis and wrong treatment of a pathological entity. The aim of this study was to evaluate the frequency of various artifacts found in oral and maxillofacial histopathologic sections.

Materials and Methods: In this cross-sectional study, the specimens included the histopathologic sections along with their diagnosis that were collected from the archive of Isfahan Oral and Maxillofacial Pathology Department using systematic sampling method over a 10-year period. These histopathologic sections were studied by two oral pathologists and an expert laboratory technician for the presence or absence of various artifacts, and the specimens from inside and outside the university were compared. The data were analyzed by SPSS software using independent *t*-test at significance level $\alpha = 0.05$.

Results: From among 237 specimens studied, 235 specimens (99.15%) had artifacts and two specimens had no artifacts. From among 21 different types of artifacts, folding ($n = 158$) and throughout cleft ($n = 149$) artifacts had the highest frequency. There was no significant difference between the specimens of inside and outside the university ($P = 0.125$).

Conclusion: The results of this study showed a high number of artifacts in the histopathologic sections, the most frequent artifact being reported for the folding artifact. It seems adequate control of specimens and preventing technical errors can reduce the number of artifacts.

Key Words: Artifact, frequency, histopathological

INTRODUCTION

Artifacts are artificial or alternative structures at the microscopic section that are made due to external factors and are observed during the examination of histological slides.[1] Artifacts may be created during a biopsy, tissue fixation, tissue preparation process, and histological sectioning and staining.[2]

Biopsy is removing a part of the tissue of a living thing for microscopic and diagnostic analysis.[3] The biopsy procedures in oral lesions include excisional, incisional, aspiration, cytology, and punch biopsy, and the use of each which depends on the size, type, and site of the lesion. It seems that, due to the small size of most oral lesions and rapid dehydration of tissue, artificial changes are more probable to occur in these samples, and oral pathologists encounter more diagnostic challenges.[4]

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Bernstein[5] reported that the artifacts due to crush using punch biopsy occur less than those caused by the surgical blade.

The artifacts caused by tissue fixation, including sample lysis occur due to lack of fixation in 10% formalin. Placing the biopsy sample in this solution immediately prevents the autolysis of sample due to the fixation of tissue proteins. If the sample is placed in the solutions other than 10% formalin, such as water, serum, and normal saline, the lysis of these solutions occurs and exerts adverse effects on the morphologic structure of the tissue, complicating the ultimate diagnosis consequently.[6] The artifacts resulting from tissue preparation process and sectioning involves folding thick sections and contamination.[7] The artifacts due to histological staining can also include stain deposit and unstained areas due to incomplete dewaxing.[8]

Seify *et al*.[9] in a similar study reported the minimum artifacts for the formalin deposition and contamination. In their study, Seoane *et al*.[10] reported a higher frequency for crush artifact and hemorrhage. In another study, the authors showed the effect of biopsy preparation on the frequency and types of artifacts.[11] In spite of artifacts' importance in histopathologic sections and scarcity of studies in this regard, it seems necessary to evaluate the histopathology of various artifacts. This is useful to identify the factors creating them and also reduce the diagnostic errors consequently. Hence, the present research was an attempt to study the various artifacts in histopathological slides of Isfahan oral and maxillofacial pathology department over a 10-year period (2003–2012).

MATERIALS AND METHODS

This study has been approved by resaerch and ethics committe of isfahan university of medical sciences, dental school (No: 394841). In this cross-sectional descriptive-analytic study, oral and maxillofacial histological slides prepared by hematoxylin and eosin staining were collected from the archive of Isfahan Oral and Maxillofacial Pathology Department from 2003 to 2012 (in this period, we had a professional technician, but after that we had a new Amateur technician in our department, so we could not evaluate the slides after 2012 because of technician variety). The specimens were collected from the archive using systematic sampling with clinical information and histopathological diagnosis. The histopathologic sections were evaluated by two oral and maxillofacial pathologists and an experienced laboratory technician for the presence or absence of various artifacts such as the stain deposition, cleft, folding and crush artifacts, tangential artifact, a raised out of focus, intralesional injection, throughout cleft, poor quality mounting, mechanical trauma, bubbles under sections, section lifting, hemorrhage, cross-contamination, thick sectioning, pink nuclei, incomplete dewaxing, unknown contamination, tissue tearing, surface cells of technician's hands or gloves, and knife lines (chatter). Then, the specimens inside the university and those sent from the outside university were compared. Finally, the obtained data were analyzed by SPSS statistics software (version 20; SPSS Inc, IBM, Armonk, NY, United States of America) using independent *t*-test at significance level $\alpha = 0.05$.

RESULTS

In this study, a total of 237 specimens (120 from within university and 117 sent from outside university) were investigated, from which 235 (99.15%) had artifacts, and two (0.85%) had no artifacts. From among the 21 types of artifacts, folding artifacts (n = 158) and throughout cleft (n = 149) had the highest frequency. In samples inside the university, folding artifacts (n = 83) and throughout cleft (n = 69) had the maximum and minimum frequency, respectively. As for the samples outside the university, throughout cleft (n = 80) and folding artifacts (n = 75) had the highest frequency, respectively [Figure 1].

The mean number of artifacts in the samples inside university was higher than that of those sent from outside university, showing no significant difference between the two groups as reported by independent *t*-test (P = 0.125).

Moreover, the frequency of various artifacts in specimens inside the university in each year was analyzed. The results indicated the maximum mean number of artifacts (4.0) in each specimen for the years 2003 and 2009 and the minimum mean number of artifacts (2.06) for the year 2012.

The results of independent *t*-test revealed no significant difference between the two groups (specimens inside and outside the university) regarding the mean number of artifacts (P = 0.12).

DISCUSSION

Artifacts or artificial structures at microscopic section

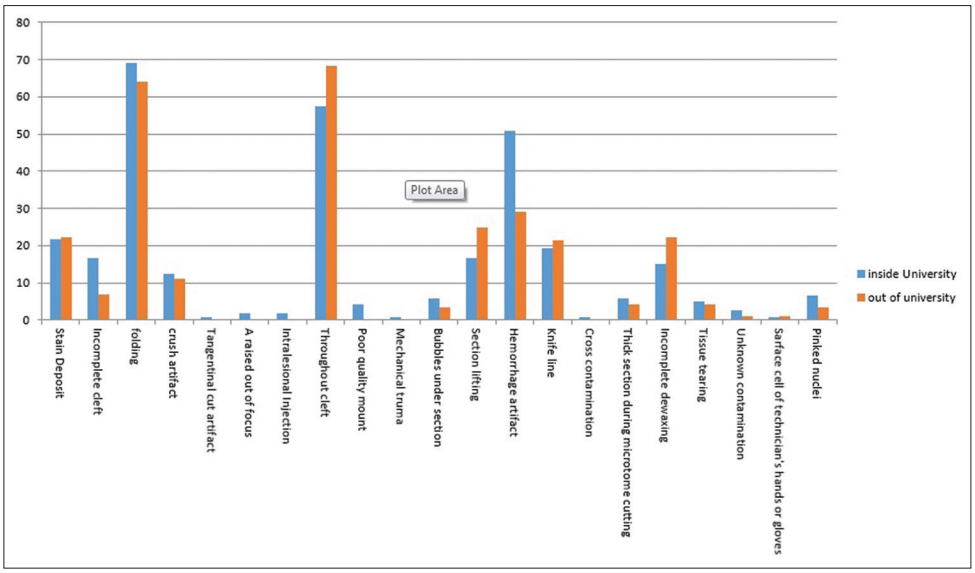

Figure 1: Frequency (%) of artifacts in the samples inside and outside the university.

cause the replacement of natural morphology and change of cellular characteristics and may even lead to diagnostic complications by pathologists.[12]

Artifacts may occur during different stages of sample preparation, including collection and transfer of samples (such as crush artifact, intralesional injection, and hemorrhage), inadequate fixation, sample preparation, improper waxing (cleft and tissue tearing), cutting the sample (knife lines [chatter] and thick sections), tissue floating (cross-contamination, folding, and section lifting), drying the samples (a raised out of focus), staining (pink nuclei, incomplete dewaxing, and stain deposition), and mounting (crystallization and bubbles) [Figure 2].[13]

In a similar study by Seify *et al*. in North of Iran, the most frequent types of artifacts were reported for throughout cleft and hemorrhage.[9] Furthermore, in the present study, folding (66.7%) and throughout cleft (62.9%) artifacts had the highest frequency. The high frequency of throughout cleft artifacts might be indicative of insufficient accuracy or haste in placing the specimens in paraffin blocks.[12] Further, the effect of local anesthetic on the tissue can be observed as vacuoles in the connective tissue.[14]

Seoane *et al*.[10] reported the crush and hemorrhage as the most frequent artifacts, and the least frequent ones to be fragmentation and throughout cleft. In the present study, throughout cleft (62.9) artifact had the highest frequency after folding artifact. The discrepancy between the results of Seoane and the current study might be due to the difference in the type of classification for analysis of artifacts resulting from the performance of oral and maxillofacial surgeons and general dentists as well as the difference in the sampling method and the study sample.

Moreover, Camacho Alonso *et al*.[15] showed the highest frequency for the folding, cleft, and hemorrhage artifacts. Considering the possible causes for hemorrhage artifact, i.e., injection into blood-rich tissue, the feedback is given to surgeons and clinicians can be helpful in decreasing this type of artifact.[15] As for the artifacts caused by pressure, for instance, use of hemostat during biopsy can lead to displacement of cells and connective fibers as well as their placement perpendicular to the pressure.[9] This squeeze can make the cells elongated and spindle-shaped (crush artifact) in this region, too.[16]

Some studies have shown the effect of oral lesion diagnosis on the presence of some specific type of artifacts.[9,17,18] Bernstein[5] reported that crush artifact is a type of change in the appearance of tissue, that is, created by the slightest pressure on the tissue, leading to chromatin extraction form the cell nucleus. Inflammatory and tumor cells are the most sensitive cells in this theory.

In their study, Seoane *et al*.[10] indicated that the biopsied inflammatory tissues were more inclined to have crush artifact than other tissues. Kontogianni *et al*.[19] reported a higher frequency for crush artifacts in malignant (carcinoma) and inflammatory specimens than in other lesions. Following the diagnostic

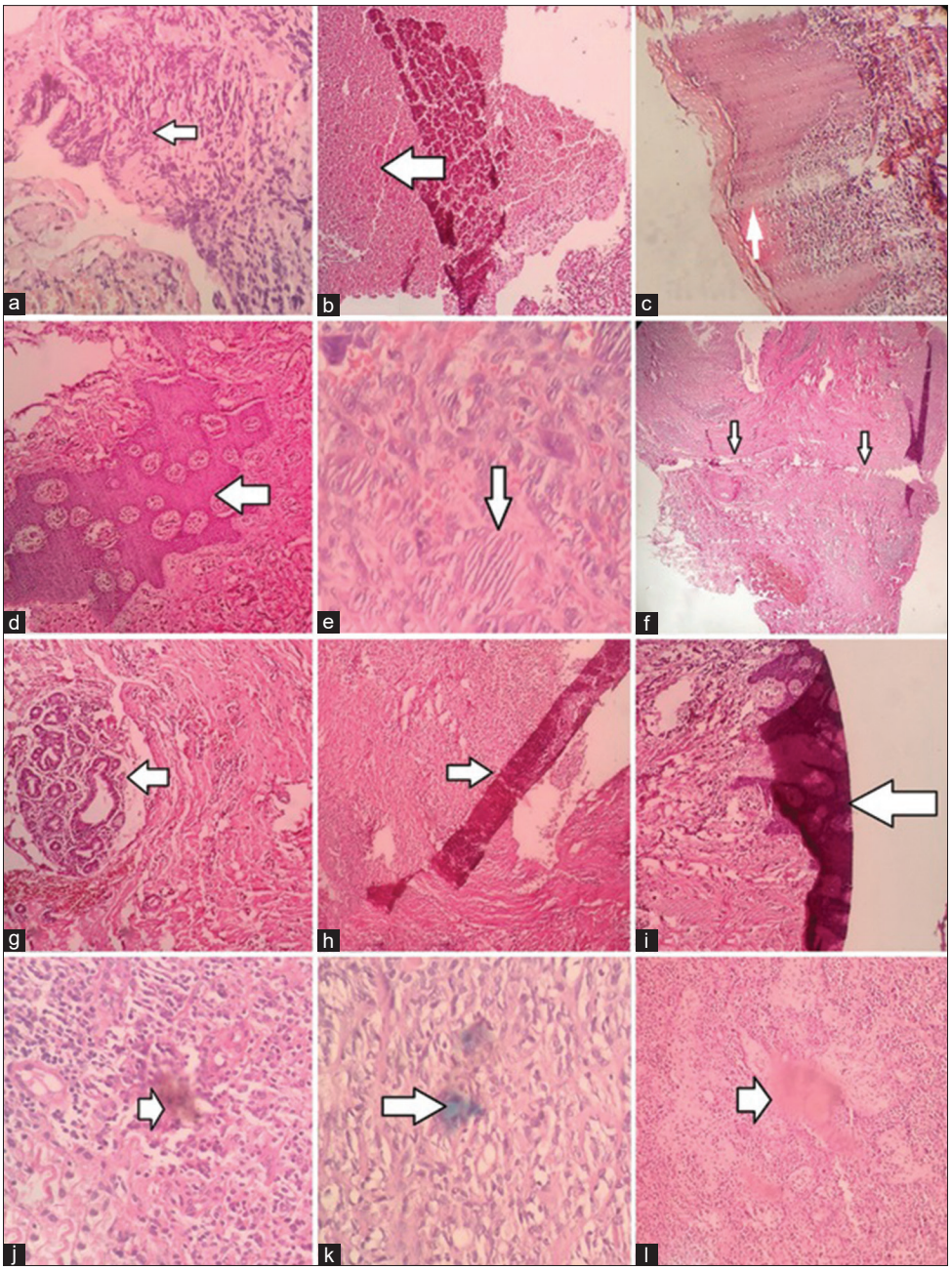

Figure 2: Microscopic view of some of the artifacts. (a) Crush artifact. (b) Bleeding artifact. (c) Incomplete dewaxing (d) Tangential cut artifact (e) knife line (f) throughout cleft (g) cross-contamination (h) section lifting (i) Folding (j) Unknown contamination (k) Surface cell of technician hand (l) bubble.

analysis of lesions and type of artifact (without exact data analysis and comparison) in the present study, a higher percentage of specimens with this artifact was the inflammatory lesions.

Folding artifacts had a higher prevalence in the current study, which could be avoided by transferring the sections to a new water bath or adding a slight amount of detergent to the water bath.[20,21] Some studies have considered the use of sharp point forceps than dull-point forceps to be effective in avoidance of overlapping the connective tissue cells.[3] Furthermore, using dull-point blades in a microtome to cut the fixed tissues can lead to overlapping tissue and folding artifact consequently.[11]

The occurrence of artifacts in specimens is unavoidable. For example, cholesterol cleft is created in the radicular cyst or periapical granuloma due to fat dissolution during processing, and lacunar cells are created in one of Hodgkin's lymphomas due to the absence of formalin stabilizer.[11,22]

The limitations of the present study were the unavailability of some histopathological slides and incomplete documents of some patients, which disturbed the comparison of diagnosis with the type of artifact. Educational pamphlets are suggested to be presented to all people during the sampling process, sample preparation, and histopathological diagnosis to introduce these artifacts and involved in minimize them with these strategies.

CONCLUSION

The findings of this study showed a high percentage of artifacts at histopathologic sections to be associated with technical and human errors. The highest frequency was reported folding artifact, which could result in distorted and unrecognizable tissues. It seems that accurate and proper preparation of specimens, prevent the mentioned errors and can reduce the incidence of some artifacts.

Limitation

due to technician variety in this period, we could not evaluate the archive after 2012.

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Conflicts of interest

The authors of this manuscript declare that they have no conflicts of interest, real or perceived, financial or non-financial in this article.

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