Scientific Research Publishing

Advances in Bioscience and Biotechnology, 2024, 15, 174-183
https://www.scirp.org/journal/abb
ISSN Online: 2156-8502
ISSN Print: 2156-8456

Common Artifacts and Remedies in Histological Preparations

Marwa Jamal Hussain Al-Kinani

College of Medicine, University of Sumer, Al-Rifai, Iraq
Email: marwa.jamal@gmail.com

How to cite this paper: Al-Kinani, M.J.H. (2024) Common Artifacts and Remedies in Histological Preparations. *Advances in Bioscience and Biotechnology*, **15**, 174-183.
https://doi.org/10.4236/abb.2024.153012

Received: January 3, 2024
Accepted: March 15, 2024
Published: March 18, 2024

Abstract

The importance of the accuracy of preparing biological specimen as histological sections that can be examined under a microscope lies in reflecting a true image of the tissue that includes all its components, which are used in scientific research or for the purpose of diagnosing various diseases of the body. Despite this, some cellular structures within the tissue may suffer from some alterations that result from the appearance of defects during any stage of preparing these microscopic sections, which alter or interfere with the precise cellular structures and morphology that constitute the tissue and thus give a different image for tissue features and cause confusion in the work histopathologist in the diagnosis. There are several reasons that can cause a misdiagnosis of the sample that occurs during the surgical separation process or after separation during the stages of microscopic preparation techniques from fixation stage, tissue processing, embedding or microtomy, staining until mounting procedures. The constant need to identify these defects and their causes in addition to try to reduce them is one of the biggest challenges evident in pathology laboratories. Therefore, this study aims to review the most common defects that occur in any stage of tissue processing, with an explanation of their causes and appropriate ways to avoid them.

Keywords

Artifacts, Histological, Preparations

1. Introduction

The precise diagnosis of numerous diseases microscopically involves preparation of stained tissue sections that reflects a natural picture as true as possible of the cellular components within the tissue during life. Experience and accuracy in the laboratory represent one of the most important of the basic requirements for

preparation of high quality sections. Most often, pathologists encounter sections that are either incorrectly fixed or mishandled during tissue processing, resulting in changes in tissue details [1]. Such alterations are categorized as "artifacts". In histological terms, an artifact can be defined as being any structure or feature that has been formed by the processing of a tissue and not normally present in the living tissue that considers a major source of diagnostic problem [2]. Some changes can be simple and the specialist can easily distinguish them from healthy or diseased cellular components of the tissue because they can change a small part of the sample and do not cause any interference with important tissue details and do not cause confusion in the diagnosis. Normal cellular or some pathological changes, and although these changes are small, they can cause a miss prognosis [3]. So in order to avoid misdiagnosis, it is necessary to understand and distinguish these defects and know their causes in order to know the appropriate solutions or avoid them. Thus, this article aims to shed light on the most common defects that face histopathologists and how to distinguish in order to prevent or at least reduce the impact of the error in diagnosis of different lesions.

2. Pre-Fixation Artifacts

It includes artifacts that may appear on section before fixation. It is usually produced due to the damage resulting from the knife or crush. Also may include tissue contamination during surgery or during tissue separation. Maintaining the tissue from any contaminated thing and accuracy of work during tissue by ensuring that all surgical instruments are washed and sterilized well, in addition to washing and sterilizing the cutting table or covering it with a clean insulating material to ensure that there is no interference with the tissue components before fixation are the best way to avoid this problem [4]. Examples of pre-fixation artifact include:

3. Presence of Sutures

Surgical suture materials are among the accidental causes of changes or deformities in the components of the tissue, and they can be composed of remains or fiber-bundles cut in any direction. Visible sutures should be removed wherever possible [5]. Tissue may contaminate with fragments of the animal's hair (**Figure 1(A)**) prior to fixation or at the time of necropsy. Such surface contaminants are often not removed by washing the tissue specimens subsequent to fixation and before to further processing, although this is the only method of removal. In some cases, depending on the orientation of the shaft of the hair, the knife can push the hair or bone further into the tissue and produce shattering of softer tissue [6].

4. Cellulose Contamination

Luminal surface of gastrointestinal tract tissues are commonly related to cellulose that may be not washed adequately before processing. Sometimes it may be

existent in an unexpected location such as within the mass of a bowel tumor (**Figure 1(B)**). It is recognized by the distinctive appearance of plant cells with their intensely staining cell walls and square shape. Cellulose may also be faced as tissue contaminant produced by the gauze used, or may have been produced due to contamination of the cutting board during sample preparation. Bathing or washing of all instruments and covering the dissection board with separate paper slips are the suitable that way avoid this problem [7].

5. Gelfoam Artifacts

Derived from absorbable gelatin, it has been used in various surgical procedures for hemostasis and can adhere to tissue surfaces (**Figure 1(C)**). Gelatin foams have a unique shape with weakly basophilic gelatin walls of varying thickness surrounded by a distorted space that may contain blood or other cell types. Normally, no tissue reaction occurs due to the presence of these substances, so in order to reduce this problem, it should to ensure that all surgical instruments used are clean and sterilized [8].

6. Starch Contamination

This defect can occur if new gloves are not washed before handling samples. Starch is used as a powder in surgical gloves so that it can be deposited in or on the surgically obtained tissue. Occasionally it may be present in surgically resected granulomas (**Figure 1(D)**). To get rid of this problem, it must make sure to wash the new gloves well before using them to reduce the presence of starch particles. Starch is difficult to see in H&E-stained sections using ordinary bright field microscopy [4].

7. Crush Artifacts

Some tissues, such as g. lymph nodes, are easily damaged when fresh by being crushed with forceps or other surgical instruments. This artifact is usually present at the periphery of the specimen, often in small localized areas such as (**Figure 1(E)**). Therefore, forceps must be used very gently and precisely and avoid pressure on soft tissues to avoid crushing them [9].

8. Tattoo Pigment

Insoluble pigments used in tattoo designs on the skin sometimes cause some changes to the morphology and components of the skin tissue (**Figure 1(F)**). These deposits are generally unreactive to histochemical tests and monorefringent under polarized light, but however, it is advised that these tattoos should be removed or reduced as much as possible to limit their retention [5].

9. Postmortem Change

Degenerative immediate alterations degenerative originate changes instantly occur tissue when is tissues are deprived of an effective blood supply. Autolysis

is created produced by releasing the release of hydrolytic enzymes through lysosomal membranes rupture. This tissue change generally shows manifests as varying degrees of nuclear pyknosis, karyorrhexis nuclear fragmentation, and karyolysis nuclear to lysis, varying accompanied degrees by along with cytoplasmic vacuolation and disintegration dissolution of tissue structure structures (**Figure 1(G)**). Glandular epithelial tissue is affected almost rapidly immediately, affected whereas while connective tissue fibers are much more resistant [10]. Microorganisms existing present in tissues postmortem can tissue may be derived from organisms which that form made up the natural flora during life (like such as those of the GI gastrointestinal tract) or contaminants from arriving postmortem contamination from various sources after death. These organisms microorganisms often are stain usually weakly stained with hematoxylin (**Figure 1**). Postmortem Storage changes are retarded by storage at 4˚C delays postmortem changes but can only be completely avoided by rapid fixation—an unlikely event occurrence in most autopsy cases [11]. Therefore, these samples must be placed in special solutions that prevent the release of hydrolytic enzymes to reduce this problem.

10. Specimen Making Dyes

India ink, silver nitrate, alcian blue are dyes that sometimes used to mark cut edges or margins of fresh or fixed biological specimens may interfere with the stains used to allow appropriate orientation of the tissue and calculation of these margins microscopically (**Figure 1(H)**). These dyes color the surface of the specimen and may also penetrate the tissue in varying degrees, therefore, it is recommended not to use color marks to cut the edges of samples and replace them with others that do not leave a color trace that could interfere with the tissue components [12].

11. Squeeze Artifact

During tissue preparation even the slight firmness of tissue by any medical tool like forceps caused distortion of tissues by caused causing hemorrhage, crush, splits, fragmentation (**Figure 1(I)**). Microscopically, reflects on the cellular details that may be not recognizable and nuclei seem darkly stained and distorted [4].

12. Specimen Contamination

This defect may occur during tissue separation where sample from a prior specimen is transported by scalpel blades or from some small pieces on a cutting board or even as tissue cassette covers (**Figure 1(J)**). To avoid these mistakes, all used tools must be washed well, as well as cleaning and washing the cutting board constantly and after each sample. Another reason of specimen transfer may occur infrequently during other stages of tissue section preparation like: section flotation baths (**Figure 1(K)**) it should continually be skimmed between samples to remove any section fragments from prior specimens that may remain [13].

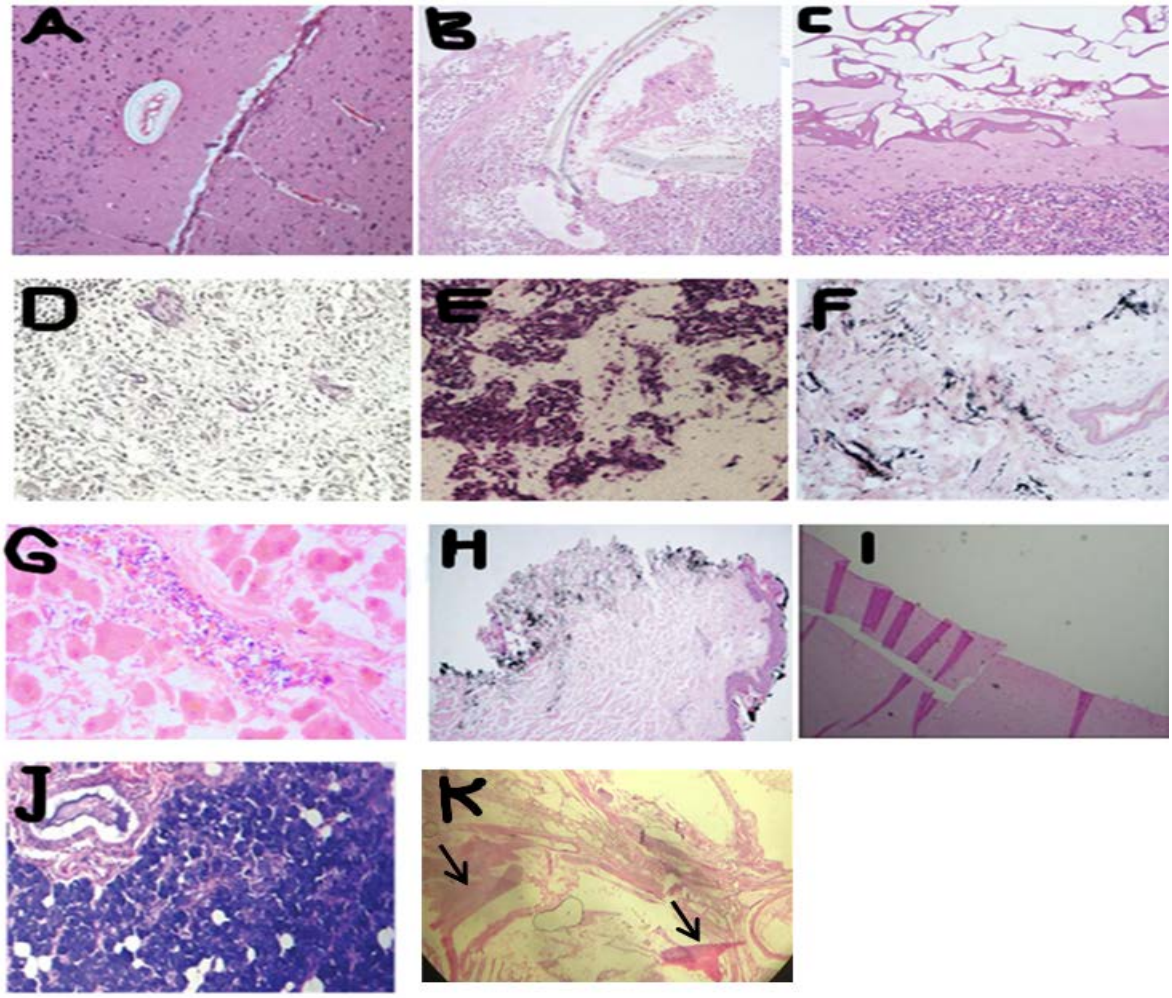

Figure 1. Histological H&E stained sections shows different type of pre-fixation artifacts: (A) A fragment of hair incorporated into brain tissue, 10×. (B) Cellulose contamination, 10×. (C) Gel foam associated with splenic capsule, 10×. (D) Starch contamination, 10×. (E) Crush artifact due to incorrect use of forceps, 10×. (F) Tattoo pigments as deposits of finely black granules in skin dermis, 10×. (G) Autolysis that poorly defined nuclei and imprecise cytoplasm staining due to post mortem change, 10×. (H) Low magnification view of the margin of a skin biopsy which has been marked with silver nitrate, 10×. (I) squeeze artifact appear as tear and folds artifacts caused by rough handling by forceps, 10×. (J) Crush artifact, 10×. (K) Defect tissue section due to contamination with other tissue, 10×.

13. Artifacts during Fixation

Some artifacts may observed in tissue sections as a result of incorrect fixation procedures, such as using of an incorrect fixative agent for a particular tissue and the formation of acid formalin haematin pigment (**Figure 2(B)**) and also autolytic changes due to adherence of specimen to the inner surface of the fixative container or due to inadequate quantities and duration of fixative agent (Thomson and Wallace, 2007). These artifact with caused such a focal area of autolysis detectable in the middle of the tissue (**Figure 2(A)**). To prevent all these problems must sure to choosing the appropriate fricative agent for a particular tissue with completely and adequate amount and enough duration for this procedure [9].

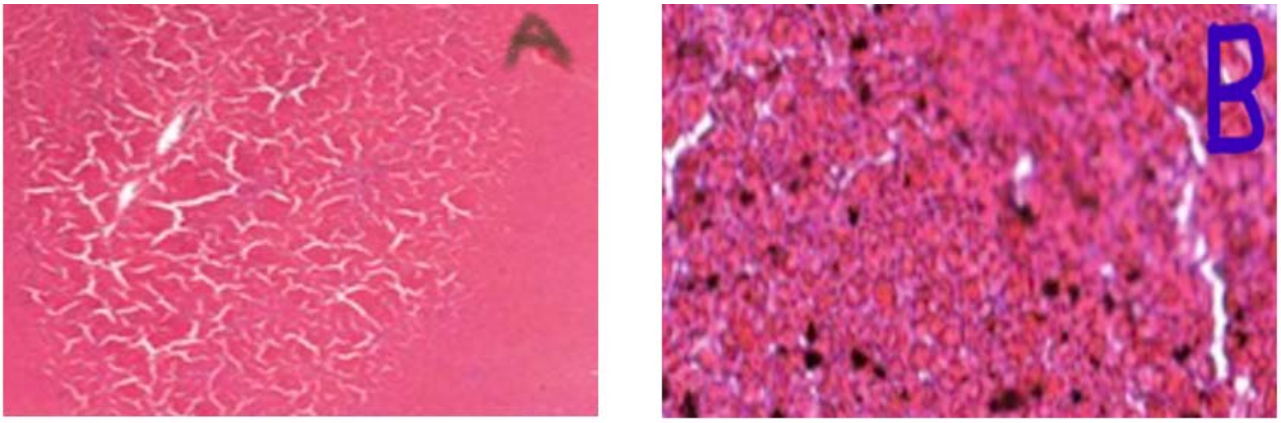

Figure 2. Histological H&E stained sections shows types of fixation artifacts: (A): micrograph shows cracking in centre of liver caused by inadequate fixation. H&E, 100×. (B): micrograph shows acid haematin formation in a blood vessel in the lung. H&E, 100×.

14. Artifacts Related to microtomy

1) Chatters

Tiny vibrations in the knife edge and excessive hardness and brittleness of the block are the main reasons of forming thick and thin zones parallel to the knife edge refers as chatters (**Figure 3(A)**, **Figure 3(B)**) To prevent these artifacts may ensure that that the cutting knife is securely installed in the holder, and also that the holder is well attached to the microtome and its necessary to decalcification of surface using sharp heavy duty knife or heavy duty microtome and finally softening the tissue [14].

2) Compression artifact:

These artifacts may include some reasons like bevel of the knife too wide, too softening of wax and also using of blunt knife (**Figure 3(C)**). In order to prevent these problems may re sharpen the knife or have regrounded, use of ice to cooling the block [4].

3) Curling artifact

Used of too soft wax produced alternate thick and thin sections also wrinkling or curling in section can occur at this step (**Figure 3(D)**). Faulty mechanism of microtome or block or blade is loose, clearance angle is insufficient. These disadvantages are avoided by cooling the block; blade tightened and raises the clearance angle [15].

15. Artifacts during Staining

Dyes that used in staining step in an appropriate way caused some defects or artifacts in tissue sections in such a way that may altered the intensity and nature of staining, due to several reasons like used of an old dyes, decomposed dyes, impurities present in the dye and leaching of certain substances from tissues into the dye (as is seen by weak staining of calcium by alizarin red S, resulting from loss of calcium ions into aqueous fixative). These artifacts can be prevented by

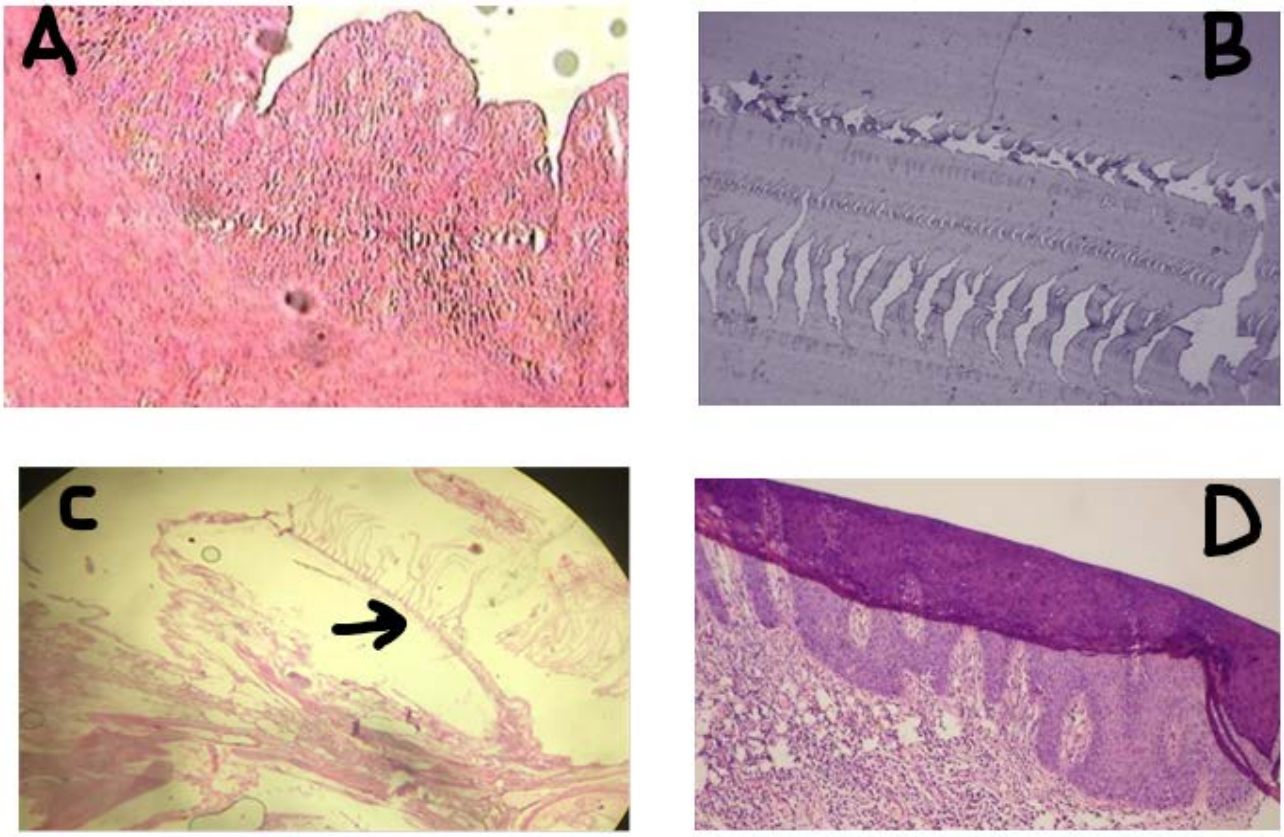

Figure 3. Histological H&E stained sections shows different type of artifacts related to microtome. (A) & (B): shows scoring and tearing of section due to nick in knife edge, 10×. (C): Displacements of fish gill filament during microtomy in due to using of dull knife, 10×. (D): curling effect due to microtome, 10×.

using ideal temperature and time, depending on the type of stain used and filtration of the staining solution that aid in remove the impurities from the stain. Slides were stained on contaminated strainer can also cause some artifacts were assessed microscopically that appears as fragments in different size on the tissue section as in (**Figure 4(A)**) [13].

Incomplete removed of wax from sections due to short duration with zylol my caused blotching of sections (**Figure 4(B)**). Improper clearing of the wax may result in undue staining of the slide, making diagnosis difficult [11].

16. Floatation, Mounting and Cover Slipping Artifacts

Some artifacts can occur when the thin sections are unevenly stretched around the rest of the structures which have different consistencies which produced dark stained strands refers as wrinkles and folding artifacts (**Figure 5(A)** & **Figure 5(B)**). These folds can be eliminated via stretching the tissue through water path and gentle drumming of remains with forceps [16]. Another artifacts appear in this stage include appearance of bubbles beneath the sections (**Figure 5(C)** & **Figure 5(D)**) when the mounting media is too thin, some bubbles may form under the cover slips [13].

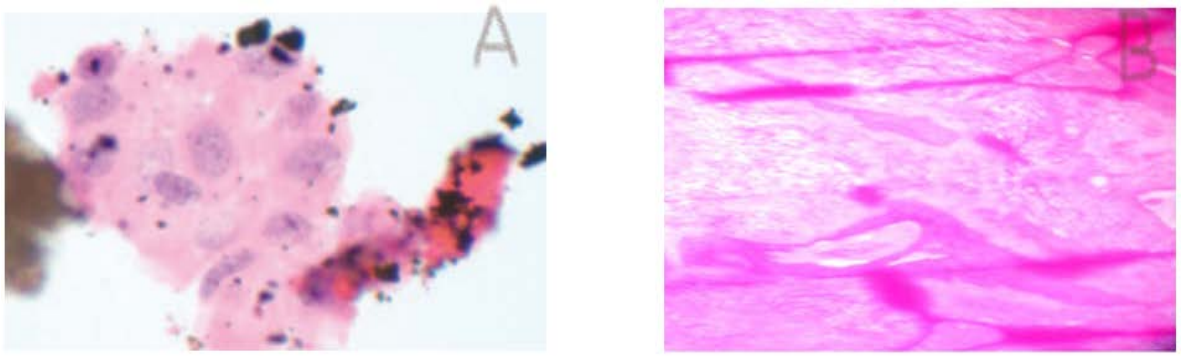

Figure 4. Histological H&E stained sections shows different type of artifacts related to staining. (A): shows contamination with fragments during strainer path, 10×. (B): shows improper wax clearing resulting in undue staining, 10×.

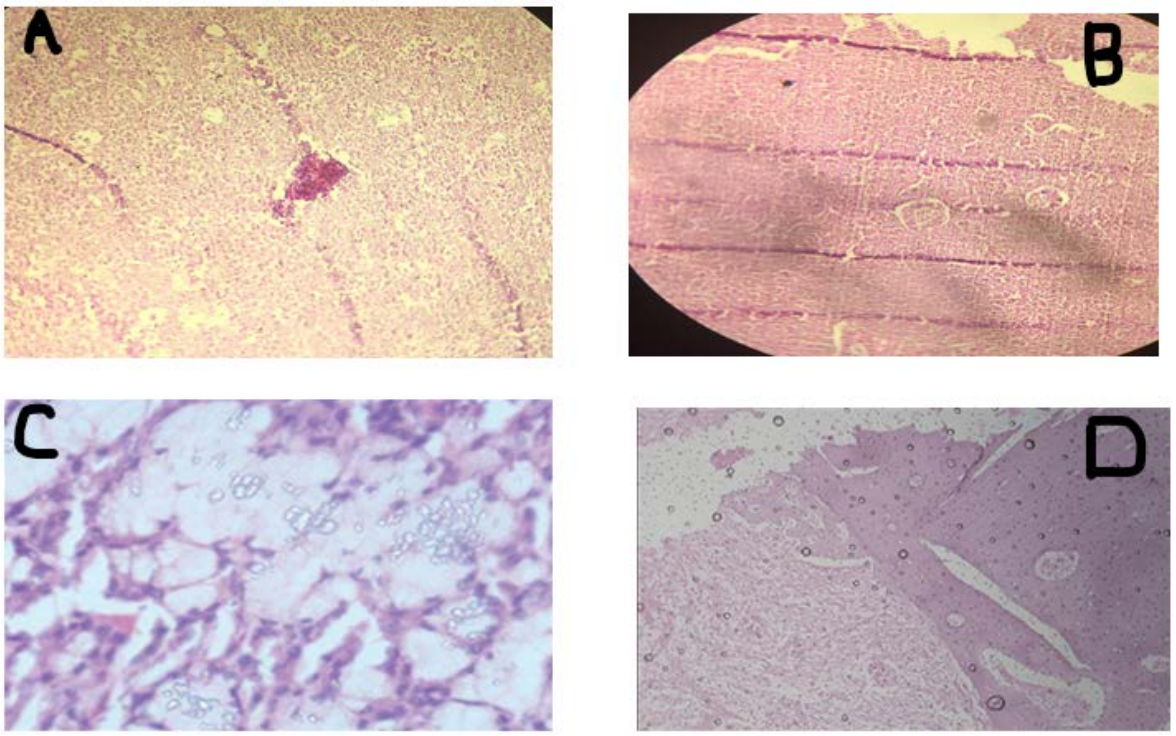

Figure 5. Histological H&E stained sections shows artifacts related to floatation, mounting and cover slipping. (A & B): fold and wrinkled artifacts related to floatation and mounting appears as dark stained strands, 10×. (C & D): artifacts related to cover slipping appears as bubbles formation due to thin mounting media, 10×).

17. Conclusion

Different kinds of artifacts can be presented into tissue sample through any preparing steps until examined by the pathologist. Various defects result in simple or multiple changes of normal morphologic or cytological features which leads to interfering or obscuring the interpretation of histopathological diagnosis, so through this article, I have illustrated some of the chief artifacts that prevent correct diagnosis as well as suggested some techniques of minimizing these problems according to the type of these artifacts. Hence, careful and appropriate handling of tissue beside the clean and sterilize of all tools used for any stage of

histological preparation, including surgical tools, gloves, and cutting boards, in addition to avoid pressing the forceps on soft tissues and using them gently and carefully to avoid tearing the samples was appropriate way to reduce all pre fixation artifact. Enough fixations and careful tissue processing will diminish these changes as much as possible in thus avoided of misdiagnosed caused by fixation and other steps.

Conflicts of Interest

The author declares no conflicts of interest regarding the publication of this paper.

References

[1] Woods, A.E. and Ellis, R.C. (Editors) (1994) Laboratory Histopathology. A Complete Reference. Churchill Livingstone, Edinburg.

[2] McInnes, E. (2005) Artefacts in Histopathology. *Comparative Clinical Pathology*, **13**, 100-108. https://doi.org/10.1007/s00580-004-0532-4

[3] Samar, K., Manisha, T., Megha, J. and Ami, D. (2014) Artifacts in Histopathology: A Potential Cause of Misinterpretation. *RRJDS*, **2**, 23-31.

[4] Bindhu, P., Krishnapillai, R., Thomas, P. and Jayanth, P. (2013) Facts in Artifacts. *Journal of Oral and Maxillofacial Pathology*, **17**, 397-401. https://doi.org/10.4103/0973-029X.125206

[5] Jimson, S., Malathi, L., Kailash, G., Kumar, G. and Balachander, N. (2016) Artifact in Histological Section. *Biomedical & Pharmacology Journal*, **9**, 843-845. https://doi.org/10.13005/bpj/1014

[6] Thompson, S.W. and Luna, L.G. (1978) An Atlas of Artifacts Encountered in the Preparation of Microscopic Tissue Sections. Springfield, Illinois.

[7] Cwati Choudhary, S., Saxena, A., Abhishek, B., Gulati, G., Khare, A. and Prasad, K. (2016) Artefact & Classification. *Saudi Journal of Medical and Pharmaceutical Sciences*, **2**, 141-145. https://doi.org/10.21276/sjmps.2016.2.6.4

[8] Seoane, C. and Ramirez, J. (2004) Artefacts in Oral Incisional Biopsies in General Dental Practice: A Pathology Audit. *Oral Diseases*, **10**, 113-117. https://doi.org/10.1111/j.1354-523X.2003.00983.x

[9] Worsham, M.J., Wolman, S.R. and Zarbo, R.J. (2001) Molecular Approaches to Identification of Tissue Contamination in Pathology Sections. *The Journal of Molecular Diagnostics*, **3**, 11-15. https://doi.org/10.1016/S1525-1578(10)60643-5

[10] Khan, S., Tijare, M., Jain, M. and Desai, A. (2014) Artifacts in Histopathology: A Potential Cause of Misinterpretation. *RRJDS*, **2**, 23-31.

[11] Pattari, S.K. and Dey, P. (2002) Facts about Artefacts in Diagnostic Pathology. *Indian Journal of Pathology and Microbiology*, **45**, 133-136.

[12] Rolls, G.O., Farmer, N.J. and Hall, J.B. (2012) Artefacts in Histological and Cytological Preparations. Leica Biosystems Pty Ltd., Melbourne, 106.

[13] Platt, E., Sommer, P., McDonald, L., Bennett, A. and Hunt, J. (2009) Tissue Floaters and Contaminants in the Histology Laboratory. *Archives of Pathology & Laboratory Medicine*, **133**, 973-978. https://doi.org/10.5858/133.6.973

[14] Culling, C.F. (1963) Handbook of Histopathological Techniques. 2nd Edition, Butterworth and Co (Publishers) Ltd., London, 123.

[15] Chatterjee, S. (2014) Artefacts in Histopathology. *Journal of Oral and Maxillofacial Pathology*, **18**, 111-116. https://doi.org/10.4103/0973-029X.141346

[16] Hunt, J.L., Swalsky, P., Sasatomi, E., Niehouse, L., Bakker, A. and Finkelstein, S.D. (2003) Microdissection and Molecular Genotyping Assay to Confirm the Identity of Tissue Floaters in Paraffin-Embedded Tissue Blocks. *Archives of Pathology & Laboratory Medicine*, **127**, 213-217. https://doi.org/10.5858/2003-127-213-MAMGAT