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Artifacts in routine histopathology: Unveiling potential causes of misdiagnosis

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Abstract

Background: The presence of artifacts in histopathologic sections can significantly contribute to misdiagnosis and inappropriate treatment of pathological conditions. The aim of this study was to evaluate the prevalence of artifacts observed in histopathologic sections and identify their underlying causes to prevent their recurrence.

Materials and methods: A total of 300 consecutive hematoxylin and eosin-stained sections were collected from the archives of the University Hospital of Mongi Slim La Marsa. These sections were thoroughly examined under a light microscope to identify the presence of artifacts.

Results: Out of the 300 slides analyzed, 273 (91%) exhibited artifacts, while 27 slides (9%) were artifact-free. The most common artifact observed was the folding artifact, accounting for 72.52% of the cases.

Conclusion: Our study demonstrated a high incidence of artifacts in the examined microscopic slides. The folding artifact was the most frequently encountered. To minimize the occurrence of these artifacts, it is crucial to implement appropriate technical measures and establish regular quality control protocols for tissue processing and staining. Pathologists must be knowledgeable about these artifacts and develop the ability to recognize them to avoid misinterpretation.

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Introduction

In daily practice, pathologists frequently encounter slides that have been inadequately fixed or mishandled during the tissue processing stage, resulting in significant alterations in tissue details. The processing of specimens for histopathological examination is prone to both material and human errors, which can give rise to artifacts. These artifacts can cause distortions in normal morphologic and cytologic features, and in severe cases, render the tissue completely unusable [1,2]. Recognizing and understanding these artifacts on microscopic slides is of utmost importance to prevent misdiagnosis and ensure accurate interpretation. Since Zegarelli published an article in 1978 addressing common problems in the biopsy procedure [3], very few additional studies have been conducted on this subject. Consequently, there remains a critical need to expand our knowledge in this area. Therefore, the primary objective of the present study is to focus on identifying specific artifacts encountered in histopathological slides and elucidating their

potential underlying causes. By doing so, we aim to develop a comprehensive understanding of these artifacts and implement preventive measures to minimize their occurrence. By highlighting the various artifacts and their causes, this study aims to fill the existing gap in literature and provide valuable insights for pathologists, enabling them to identify and mitigate these artifacts during the tissue processing and slide preparation stages. Ultimately, this research endeavors to enhance the accuracy and reliability of histopathological diagnoses, thereby improving patient care and treatment outcomes.

Methods

Study Design: This descriptive cross-sectional study involved the analysis of 300 consecutive histopathological slides obtained from the archives of the University Hospital Mongi Slim La Marsa. The examination of the slides was carried out by a pathologist and a laboratory technician, utilizing a light microscope at magnifications of ×4, ×10, and ×40. The objective of the study was to assess the presence or absence of various artifacts that may arise during tissue processing, from fixation to slide mounting. This microscopic examination aimed to provide insights into the prevalence and characteristics of artifacts observed in the histopathological slides, thereby contributing valuable information for understanding and addressing potential sources of error in tissue processing and slide preparation.

Inclusion and exclusion criteria: Three hundred formalin-fixed and paraffin-embedded histological tissue slides processed in the Department of Pathology of Mongi Slim Hospital La Marsa between May 2023 and October 2023 were included in this study. However, slides that were broken or improperly labeled were excluded from the analysis.

Artifact assessment: The histological tissue sections were meticulously examined using a Leica DM500 microscope to identify various patterns of artifacts. These artifacts were characterized as artificial structures or alterations observed on the prepared slides. The aim of this assessment was to determine the presence and nature of artifacts, providing valuable insights into the potential challenges and pitfalls associated with tissue processing and slide preparation procedures.

Results

In this study, a thorough investigation was conducted on a total of 300 microscopic slides to assess the presence of artifacts and their prevalence. The slides were meticulously reviewed and analyzed by a single experienced pathologist. Out of the 300 consecutively examined slides, 273 slides (91%) displayed the presence of various artifacts (Table 1), while, 27 slides (9%) were found to be free from any detectable artifacts. Multiple artifacts were observed in several slides, with two or more being present.

Prefixative artifacts

Within this group, split artifacts accounted for 10 cases, representing 3.66% of the total. Additionally, there were 6 cases of crush artifacts (Figure 1A), contributing to 2.19% of the total. In contrast, artifacts attributed to contaminants were observed in only 5 cases, comprising 1.83% of the total. Hemorrhagic artifacts and heat artifacts were each identified in a single case, representing 0.36% of the total for both types of artifacts.

Fixative artifacts

The only artifact observed at this stage was formalin pigment artifact which had 2 cases (0.73%).

Tissue processing artifacts

The predominant artifact pattern observed in this study was the fold artifact (Figure 1B,C), resulting from lifting tissue sections from the water bath. This particular artifact was identified in 198 cases, accounting for 72.52% of the total cases. Another notable category of artifacts, constituting 18.31% of cases, stemmed from faulty microtomy (n=50). These included knife line artifacts (Figure 1D), scratch artifacts, and crumbling artifacts.

Staining and mounting artifacts

Among the observed cases of artifacts in this stage stain deposition (Figure 2A) on the prepared histological sections (n = 4), accounted for 1.46% of cases. Additionally, 11 cases (representing 4% of the total) exhibited artifacts resulting from residual wax (Figure 2B). Furthermore, 65 cases (comprising 23.8% of the total) were associated with artifact formation due to air bubble entrapment during the mounting process (Figure 2C). Dry mounting was observed in 33 cases (12.09%). Excess mountant was identified in 13 cases (4.76%).

Lastly, 7 cases (constituting 2.56% of the total) were identified to have artifacts caused by contaminants (Figure 2D).

Discussion

Establishing a correct histopathological diagnosis relies on several fundamental requirements, including an appropriate biopsy procedure, optimal fixation and processing techniques, and accurate sectioning and staining methods. The identification and interpretation of tissular structural and morphological details are essential for accurate histological analysis [1,4]. However, the histopathological examination of microscopic slides is often hindered by the presence of artifacts, which introduce alterations in normal morphological and cytological features. These artifacts can occur at various stages of sample preparation [4,5] and are classified based on the specific phase in which they originate. Artifacts can manifest during different steps of the histopathological workflow, including fixation, tissue processing, embedding, microtomy, mounting, staining, and even the surgical biopsy procedure itself. These artifacts can vary in severity, with some affecting only a small portion of the specimen, which may not significantly impact the pathologist's ability to provide an accurate diagnosis. However, in certain cases, the artifactual damage can be substantial, rendering the specimen suboptimal or even useless for diagnostic purposes [5]. It is crucial to recognize and understand the potential sources of artifacts in histopathology to minimize their occurrence and mitigate their impact on diagnostic accuracy [5].

Types of artifacts

Pre-fixation artifact: Pre-fixation artifacts occur before tissue fixation and can include deposits like tattoo pigment, as well as artifacts from surgical procedures such as laser knife damage or crush artifacts. In our series, crush artifacts were identified in 6 slides (2.19%). Contaminants can also be introduced during surgery or specimen handling. To prevent such artifacts, ensure awareness of the potential consequences of specimen contamination or damage [6].

Fixation artifacts: Tissue fixation aims to prevent decay and preserve cells, but inadequate fixation can cause autolytic changes such as shrinkage, crenation, swelling, and bursting.

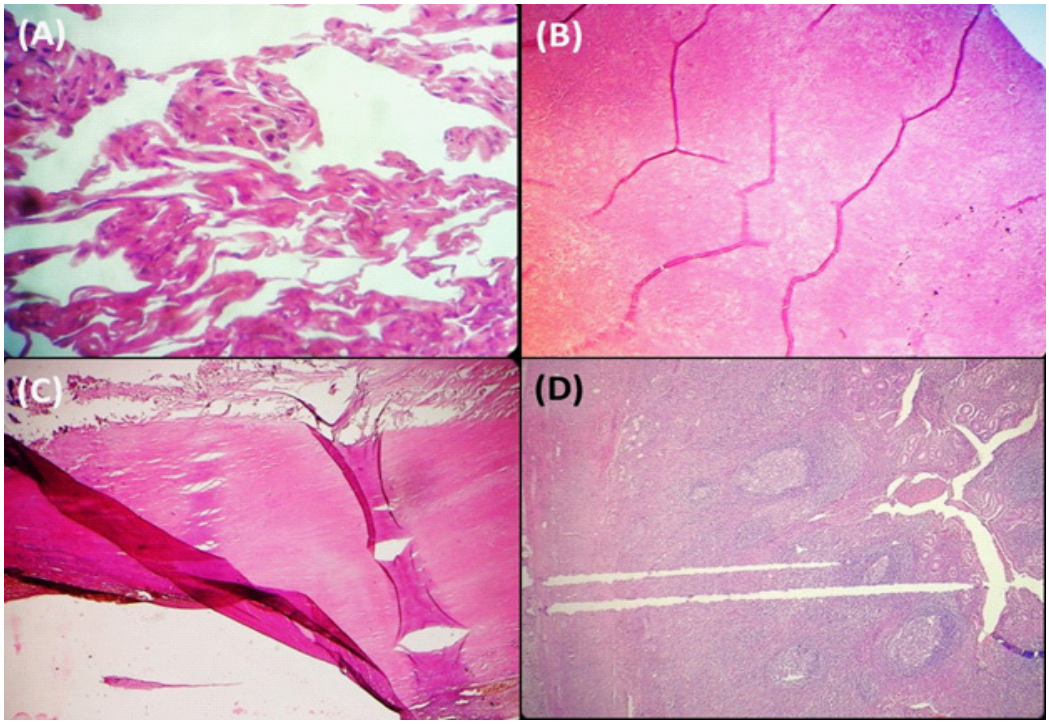

Figure 1A: Cauterization or crush artifact (Hematoxylin and eosin, magnification ×400).

Figure 1B: Histopathological image shows wrinkles and folds due to uneven stretching of tissue sections (Hematoxylin and eosin, magnification ×100).

Figures 1C: Artifact during tissue lifting: Tissue folding (Hematoxylin and eosin, magnification ×400).

Figures 1D: Knife line artifact (Hematoxylin and eosin, magnification ×100).

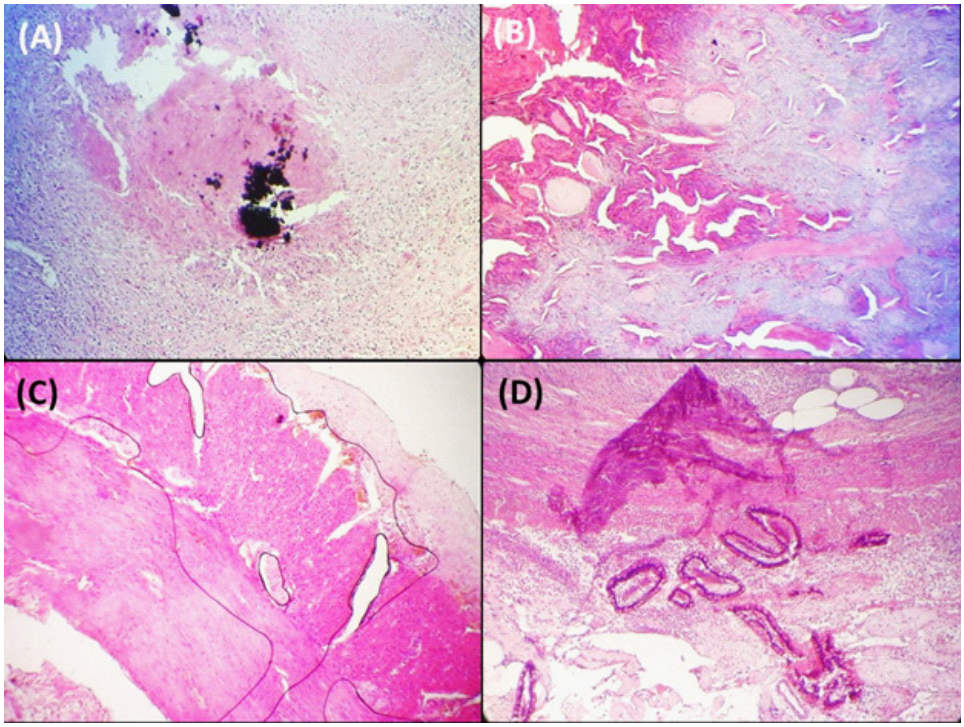

Figure 2A: Stain deposits within the histological section, (Hematoxylin and eosin, magnification ×100).

Figure 2B: Residual wax within the stained section, (Hematoxylin and eosin, magnification ×40).

Figure 2C: Histopathological image showing air bubbles formed during mounting procedure (Hematoxylin and eosin, magnification ×100).

Figure 2D: Artifact due to contamination. Specimen-specimen contamination. A section of the appendix contaminated by a duodenal biopsy, (Hematoxylin and eosin, magnification ×100).

Table 1: Types of artifacts.

Patterns	Number of slides	% of artifacts	% of total artifacts
Pre-fixative Artifacts	**N**	**% of Prefixative artifacts**	**% of total artifacts**
Heat	1	4.34	0.36
Crush	6	26.08	2.19
Split	10	43.47	3.66
Contaminant	5	21.73	1.83
Hemorrhagic	1	4.34	0.36
Total	**23**	**100**	**8.4**
Fixative Artifacts	**N**	**% of Fixative artifacts**	**% of total artifacts**
Formalin pigment	**2**	**100**	**0.73%**
Tissue processing artifacts	**N**	**% of tissue processing artifacts**	**% of total artifacts**
Fold	124	71.26%	45.42%
Scoring	50	28.73%	18.31%
Total	**174**	**100%**	**63.73%**
Staining and mounting artifacts	**N**	**% of Staining and mounting artifacts**	**% of total artifacts**
Residual wax	11	9.16%	4%
Stain deposition	4	3.33%	1.46%
Air bubble	65	54.16%	23.8%
Contaminant	7	5.83%	2.56%
Dry mounting	33	27.5%	12.09%
Total	**120**	**100%**	**43.91%**

Using a phosphate-buffered saline-based fixative can help resolve these issues. However, fixation can introduce artifacts due to suboptimal conditions and low-quality reagents [7]. Common fixatives like formalin can hinder sectioning if not used optimally, leading to brown-black or yellow stains. Prolonged fixation can result in secondary shrinkage, hardening, and separation of tissues. Additionally, fixation can cause changes in tissue size and induce swelling with non-protein precipitants [7].

Grossing and processing artifacts: Cross-contamination artifacts, known as floaters, can occur when tissue from other areas contaminates a slide during grossing, processing, or floatation of cut-sections [1]. Processing thin and narrow tissue specimens may lead to curling, posing challenges in orientation and tangential section formation. Microwave tissue processing is a technique that minimizes shrinkage and eliminates the need for formalin and xylene [8,9]. Improper adjustment of an automatic tissue processor or power failure can cause tissue dehydration, excessive staining, and section cracking [1,10]. Biopsy foam pads in embedding cassettes can produce grid-like or triangular artifacts, while incomplete dehydration can result in inadequate staining or opacity within the section. Regularly changing processing solutions and covering containers can help prevent these issues [1]. Inadequate tissue infiltration with paraffin can cause wrinkles in all directions due to fixation, dehydration, clearing, or insufficient time in molten wax [1]. Prolonged processing schedules can excessively shrink, dry, and fragment small tissues, resulting in overstained or crushed sections. Using shorter processing schedules is recommended [11].

Embedding: During embedding, the entrapment of air around the tissue can lead to the venetian blind artifact, characterized by compressed tissue zones separated by open spaces [8]. Embedding multiple tissues with different consistencies in the same block can also cause artifacts [12]. Retention of hydrophilic processing fluids within the embedded tissue block can result in wrinkled sections. Inappropriate embedding medium hardness, rapid wax cooling, contamination with clearing agents, denatured wax, or insufficient dehydration can lead to tear artifacts [7].

Microtomy/sectioning artifacts: Microtomy or sectioning artifacts can include thick and thin sections, chatter/venetian blind artifact, scratch lines, crumbling sections, creases in cut sections, and displacement of tissue components. These artifacts can result from factors such as a loosely attached microtome knife or tissue block, steep cutting knife angle, hard tissue or wax, presence of calcification, nicks in the knife edge, large knife clearance angle, hard material in wax or tissue, blunt knife or soft wax, contamination of wax, loss of bevel on the knife edge, and poor adhesion of sections to the glass slide. During a one-year period, Igho OE and Aimakhume A conducted a study where they observed a total of 406 artifacts across 388 tissue sections. Interestingly, the majority of tissue sections displayed multiple patterns of artifacts, reinforcing the complexity of artifact occurrence. Their findings revealed that artifacts were present in 94.58% of the sections, aligning with the results of the current study. Notably, similar to our findings, fold artifacts emerged as the most prevalent type of artifact [13]. Tissue folding artifacts often arise during the lifting of tissue sections. However, these artifacts can be mitigated by transferring the sections to a fresh water bath and adding a small amount of detergent to facilitate optimal section spreading [14]. Chatterjee S further supports the prevalence of fold artifacts in tissues containing hard components, emphasizing the challenge in completely avoiding them despite meticulous care [15].

Floatation and mounting: During the processing stage, artifacts can arise from various sources, including contamination by microorganisms (such as fungi), air-borne fibers, hair, cellulose fibers, and the presence of floaters or bubbles beneath the sections. Contamination from exfoliated squamous cells, caused by contact with fingers or sneezes/coughs, is also a common artifact [13]. Care must be taken during processing and floatation to prevent folding of microscopic tissue sections. Trapped air bubbles can lead to the formation of collapsed bubble artifacts, resulting in cracked areas when dry and improper adherence to the glass slide, leading to altered staining [16].

Staining: Incomplete removal of wax from sections can result in residual wax artifacts, impairing staining [1]. Stain deposits may appear if dye solutions are old or unfiltered. Precipitated eosin flakes above the focal plane of the tissue section can occur from an unfiltered stock solution [1]. Inadequate drying

between the last xylene and cover slipping can lead to minute bubbles trapped over the nuclei, resulting in dark nuclei without visible detail (corn flake artifact) [8]. Water presence in the sections masks microscopic detail and causes stain leaching. Washing eosin-stained sections in tap water with an acidic pH can result in stain leaching into the mounting media, especially in high humidity conditions [13].

Cover slipping: Bubbles under cover slips can form if the mounting media is too thin. In our series, 65 cases (23.8%) were associated with artifact formation due to air bubble entrapment during the mounting process. Decay and crystallization of mounting media may occur over time if resin-based mountants are prepared incorrectly. Prolonged exposure of sections to light can lead to unwanted bleaching of stains, emphasizing the importance of storing stained sections in dark cabinets. The use of slide holders can help prevent fingerprints on slides, and maintaining a clean and organized mounting bench can minimize contamination of tissue sections by debris, fibers, or fungi [1].

Conclusion

Our study found a high prevalence of unintended artifacts in examined microscopic slides, particularly section folding. Recognizing and addressing these artifacts is crucial for accurate diagnosis. To minimize artifact occurrence, implementing technical measures, establishing quality control protocols, and providing proper specimen handling and processing are important. Meticulous attention to detail throughout the histopathology workflow can enhance the reliability of diagnoses. Continuous education and training of laboratory personnel on artifact recognition and prevention strategies is essential. Overall, implementing meticulous techniques and standardized protocols can reduce artifacts and ensure high-quality slides for precise diagnostics.

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