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A ONE-YEAR RETROSPECTIVE EVALUATION OF HISTOPATHOLOGICARTIFACTS

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ABSTRACT

The study of tissue obtained from living things is an art that requires extreme care and practice. However, the inevitability of artifacts may result in the wrong diagnosis obtained from laboratory upshots. Despite documented treatise on the prevalence and occurrence of artifacts on histopathologic slides processed in Nigeria laboratories, its study regarding the pattern is scanty. This prompted our interest in delineating the several patterns an artifact exhibits for a year at Polyhybrid Forensic Consult. Ethical clearance was sought and obtained from the Research and Ethics Committee of Human Anatomy Department, Faculty of Basic Medical Sciences, Delta State University, Abraka, with reference no: DELSU/CHS/ANA/2021/98. Permission was also obtained from the management of Polyhybrid Forensic Consult prior to Data collection. Obtained slides were viewed under a microscope connected to a computer, and data were collected using a data collection sheet. Obtained data were analyzed statistically for percentage of occurrence and cumulative ratio using the Statistical Package for Social Sciences (SPSS) software version 20. The results were presented in frequency tables. Results depict the fold artifact as the most prevalent, with 133 cases (33.84%) and hemorrhagic artifact as the least with 1 case (0.25%). Artifacts are inevitably avoided in the tissue section, and fold artifact, which is recorded as the most prevalent in the index study, is attributed to thick tissue section, improper stretching of the section, irregular lifting of the section from the water bath and swift removal and dipping of tissue section into and out of processing reagents.

KEYWORDS: Artifacts, histopathologic, retrospective, fold.

INTRODUCTION

Artifact as a locution is coined from the Latin words art and factum. It alludes to the artificial structure or tissue shift actuated by external agents usually found on a microscopic slide.[1] In reality, artifacts lead to an alteration within the normal cytological or morphological form, which may subsequently lead to histological distortion and incorrect conclusions or outcomes.[2] Distortion of tissue as related to artifact may be incurred from tissue preparation or histologic analytical tools.[3] Artifacts happen for different reasons, and one of them is the sampling process; punch biopsy, as a subtype of sampling process, which has been shown to take off considerably fewer artifacts in contrast with incisional scalpel biopsy.[4] More or also, the degree of artifacts within the margins of CO_2 laser tests was observed to be prevalent as compared to steel instruments.[5] However, the process of fixation, which is also a part of the sampling procedure, may also lead to artifacts. This was extensively discussed in a study conducted by Abreu *et al.* where samples were not properly fixed.[6]

The gold standard for diagnosing most of the diverse pathologies is the microscopic evaluation of slides.[7] The steps taken by the specialist, their associate, and the histo-technician to downplay the chance of artifacts are connected to the satisfactory preparation of tissues for microscopic examination.[8] The precise determination of several lesions under a magnifying lens requires the preparation of tissue parts, usually stained, that reflect their life structures as closely as conceivable. The preparation of high-quality slides involves expertise in laboratory practice; however, histologic alterations are inevitable in recent practice and interfere with diagnosis.[9]

There have been several studies on histopathology artifacts in specific studies of organs worldwide. However, the histopathologic practice has limited treatizes of artifacts of variant forms. Therefore, this study sought to appraise the prevalence and patterns of artifacts in several organ studies at the poly hybrid forensic consult,

which may be useful to identify the factors creating them and avoid their inevitability in histopathology.

MATERIALS AND METHODS

Ethical Consideration

Ethical clearance was sought and obtained from the Research and Ethics Committee of Human Anatomy Department, Faculty of Basic Medical Sciences, Delta State University, Abraka, with reference no: DELSU/CHS/ANA/2021/98. Permission was also obtained from the management of Polyhybrid Forensic Consult prior to Data collection.

Study Design

This study adopts a descriptive cross-sectional study design.

Study Population

This study consists of histopathological slides retrieved from the archive of the histopathology laboratory at the Polyhybrid Forensic Consult from 2021 to 2022.

Study Area

This study was conducted in the Polyhybrid Forensic Consult, Ovu Inland, Delta State.

Data Collection

A data collection sheet was used to compile the data obtained from archived slides in the histopathology laboratory. Data were obtained by viewing the slides under the microscope (Carl Zeisss Suzhou Co., Ltd, China, with serial number 3153010787, Prime Star).

Inclusion Criteria

- Artifacts based on surgeon and technician performance were included in the study.
- Intact slides with undamaged labels were included in the study.
- Only slides within the study period were used for the study.

Exclusion Criteria

- Broken and unlabelled slides were excluded from the study.
- Repeated slides from each tissue block were excluded from the study to avoid bias.

Data Analysis

Data was analyzed statistically for percentage of occurrence and cumulative ratio by using the Statistical Package for Social Sciences (SPSS) software version 20. The results were presented in frequency tables.

RESULTS

In all slides investigated, 393 artifacts were recorded, with some displaying one or more different types of artifacts. These were represented as follows.

Table 1.

Artifacts Type	Artifact Sub Class	Frequency	Percentage of Prefixative Artifacts	Percentage of total Artifacts
Prefixative Artifacts	Hemorrhagic	1	11.11	0.25
	Squeeze	4	44.44	1.02
	Freezing	4	44.44	1.02
	Total	**9**	**100.0**	**2.29**
Fixative Artifacts	Formalin pigment	**23**	**100.0**	**5.85**
	Total	**23**	**100.0**	**5.85**
Tissue Processing Artifact	Improper Dehydration	4	2.04	1.02
	Microtome	21	10.71	5.34
	Fold	133	67.86	33.84
	Wrinkles	38	19.39	9.67
	Total	**196**	**100.00**	**49.87**
Staining and Mounting Artifacts	Residual wax	8	8.85	2.04
	Stain deposit	12	7.27	3.05
	Air bubble	46	27.88	11.70
	Mounting	57	34.55	14.50
	Staining	42	25.45	10.69
	Total	**165**	**100.00**	**41.98**

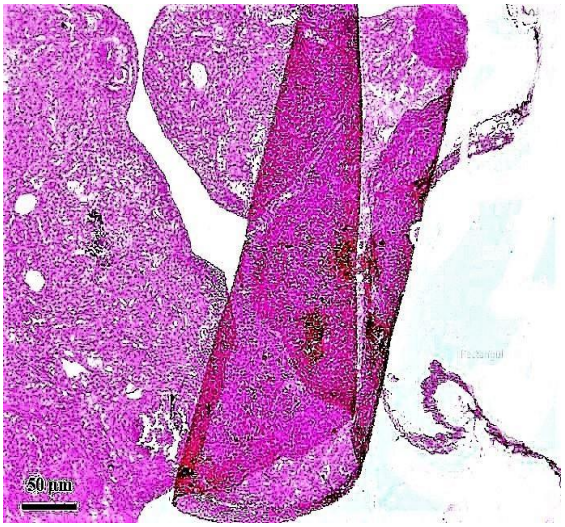
Table 1 above represents artifact type; with **prefixation artifacts**, only one case was hemorrhagic, contributing to 11.11% in this group, and four cases (44.44%) were squeeze and freezing artifacts. Prefixation artifacts' total percentage was 2.29%. For **fixative artifact**, 23 cases of formalin pigment were recorded, with an overall

percentage of 5.85%. For **tissue processing artifacts**, fold artifact was observed to be prevalent, with a frequency of 133 out of 196 cases. For this fixative type, an overall percentage was observed to be 49.87%, the highest percentage recorded in this study compared to other artifact types. However, **in staining and mounting artifacts**, staining, mounting, and air bubbles as subtypes were found to be prevalent, with a case report of 42, 57, and 46, respectively. A total of 41.98% was recorded as an overall percentage.

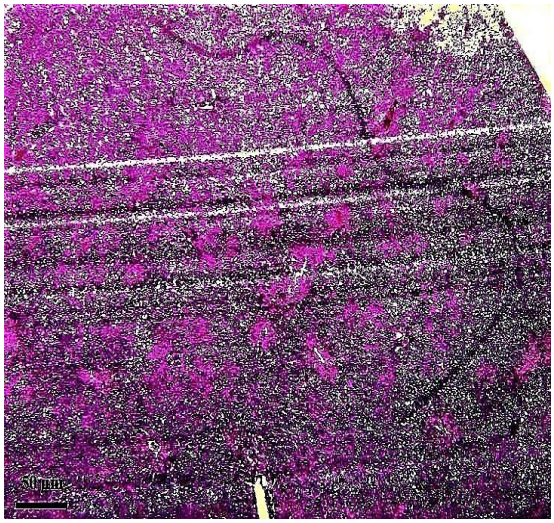
Table 2.

Patterns	Frequency	Percentage of total Artifacts
Prefixative artifacts	9	2.29
Fixation artifacts	23	5.85
Tissue Processing Artifacts	196	49.87
Staining and Mounting Artifacts	165	41.98
Grand Total	**393**	**100.0**

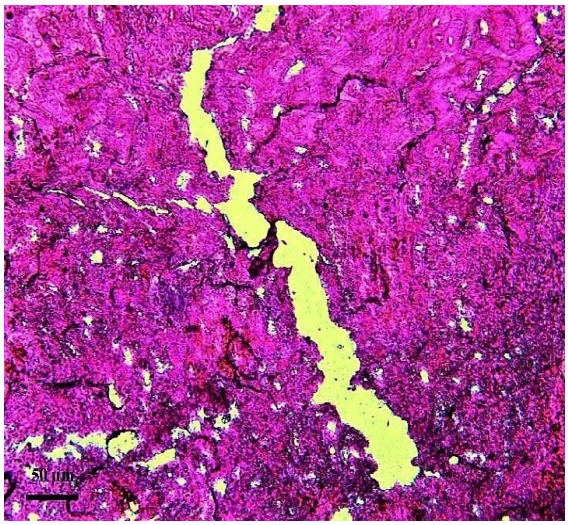
Table 2 above shows the overall artifacts examined in the study. About 196 cases, representing 49.87%, were tissue processing artifacts, followed by 165 cases of staining and mounting artifacts, representing 41.98%. Fixation artifacts had a total of 23 cases, representing 5.85%. The least was prefixative artifacts, which had only 8(2.29%) cases.



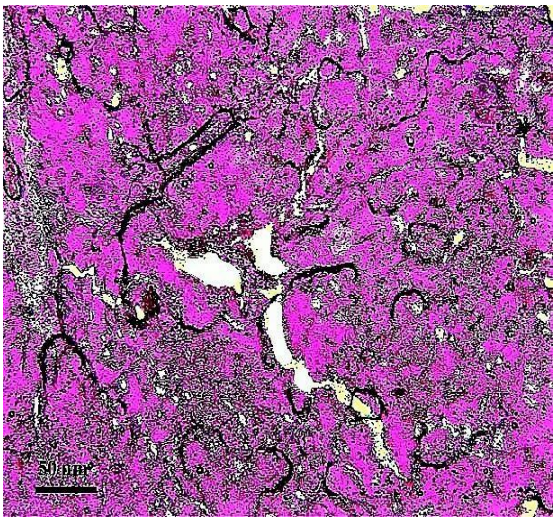
Fold Artifact



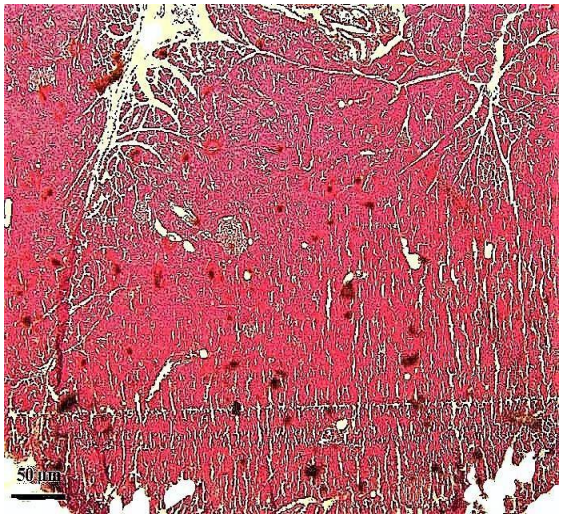
Knife Artifact



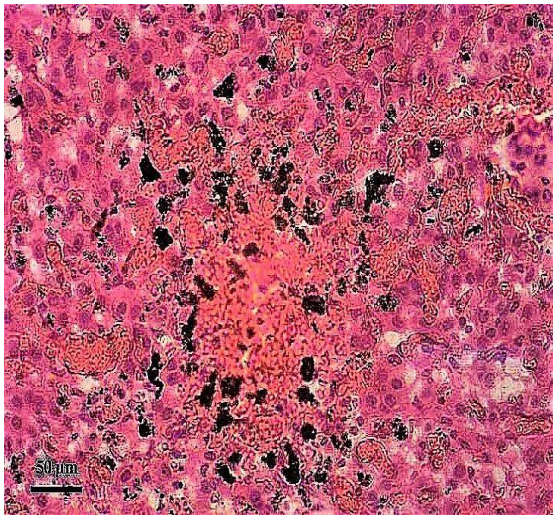
Tear/Separation Artifact



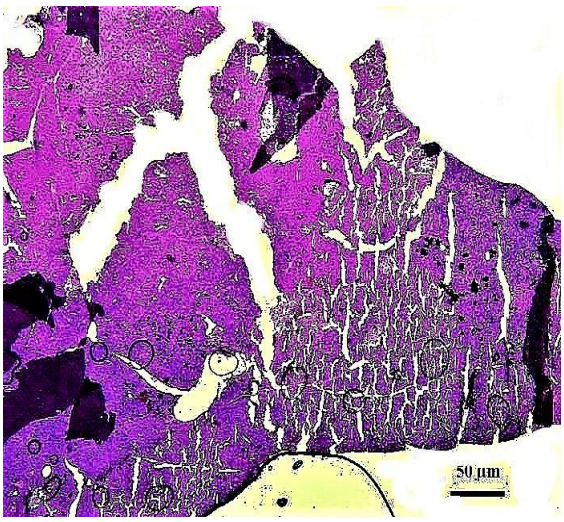
Water bubble Artifact



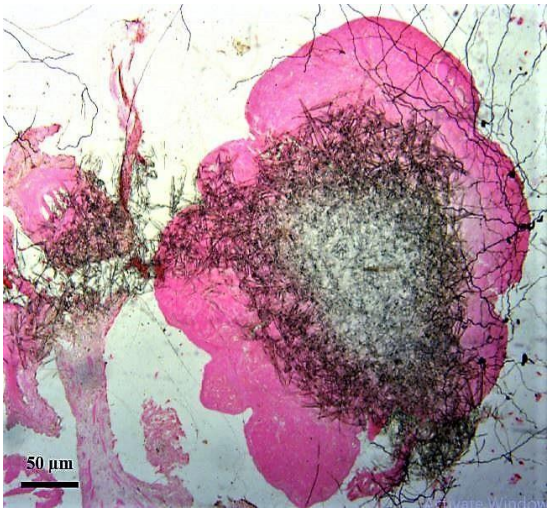
Microtome Artifact



Formalin Artifact



Mounting Artifact



Fungi growth Artifact

DISCUSSION

The upshot of the yearly hindsight study of the artifact at poly hybrid forensic consult revealed sampled slides having more than one pattern of artifact, broadly classified into three; artifact caused by surgeon, technician and sample transfer.[10] A further classification of artifacts sampled by the index study was observed in a study conducted by Taqi *et al.*, which elaborated on prefixation, fixative, tissue processing, sectioning, staining and mounting artifacts.[11] From the report recorded, fold artifact is observed to be prevalent and also believed to have resulted in the majority of the recorded cases.[12]

Artifacts incurred before fixing the tissue (prefixative artifact) are mostly observed from surgical procedures.[1, 13] This was clearly elaborated in the index study as a squeeze artifact resulting from the use of toothed forceps, where the tooth pierces the tissue causing a tear or compression of the tissue.[10, 14] Histologically, this can distort the tissue cyto-architecture, resulting in a nuclear-level shift. In the index study, squeeze artefact, which comprises crush, fragmentation, splits and hemorrhage[4, 9], was observed to be more prevalent among the prefixation artifact, and these findings are similar to a report obtained by Camacho *et al.* (2008) study, where there was an observed increase in the frequency for the folding, cleft, and hemorrhage artifacts.[15] Regarding the presence of freezing artifact, which was also prevalent as a prefixative artifact and is observed not to be a very common type of artifact. This was discussed in a study conducted by Okun *et al.* where freezing artifacts are incurred from the slow freezing of the tissue or selection of large tissue samples for rapid freezing.[16]

Fixative artifacts are artifacts that are incurred from fixing a tissue. For most cases, the index study recorded formalin pigment as a sub type of fixative artifact. These artifacts are seen around vessels composed of erythrocytes, and these cells are constituted by a pigment (hemoglobin), which is known to react with acid formalin used in preservation, giving a dark to brown opaque pigment.[17, 18] The reason, as mentioned earlier stated by Bancroft and Burns, maybe a reason for the prevalence of this artifact pattern in the index study. However, the use of other formalin diluents constituted with a regulated time

of fixation may aid the ameliorative action of these artifacts.

Tissue processing is the act of properly removing water from the tissue and replacing it with a rigid medium that supports proper sectioning without any form of distortion.[14] In processing and sectioning a tissue block, the inevitability of artifact is incurred.[11] The index study addressed fold artifact as the most prevalent tissue processing artifact, which was synonymous with a study conducted by Odokuma and Aimahafue.[12] These artifacts are incurred when tiny sectioned ribbons are compelled to stretch in a floating bath or forcefully dipped in several processing reagents.[17, 19] Fold artifact can also be incurred from microtomy procedures. This results from vibration in the knife edge, brittle nature of tissue block or excessive inclination of knife angle.[2] However, utmost care taken with respect to the floating of sectioned ribbons in water and the sectioning process has been demonstrated to minimize the occurrence of these artifacts.

Rastogi *et al.* have shown that received specimens in a histopathology laboratory have shown to be affected by variant forms of artifact, which result from several histologic processes, including staining and mounting of the tissue medium.[1] The use of stains and mountants in histologic preparation is essential and gives a better understanding of the study of tissue morphology.[3, 20] As seen in the index study, these processes are unavoidably present with residual water and air bubble artifacts, displayed prevalence as a subtype of staining and mounting artifact. This was similar to a study conducted by Rolls *et al.* and Odokuma and Aimakhume, where the drying up of tissue sections between the last xylene and coverslip resulted in the entrapment of minute bubbles over the nuclei leading to dark nuclei lacking visible detail.[12, 21] Their study also observed that air bubbles result in one-fourth of the total number of artifacts reported in tissue sections. Therefore, careful staining and mounting procedures should be strictly adhered to.

CONCLUSION

Artifacts that are inevitably present in tissue sections are a huge concern regarding histopathologic diagnosis. However, the folding of tissue sections has become a common type of artifact, which is recorded as the most prevalent in the index study. This has been attributed to thick tissue section, improper stretching of the section, irregular lifting of the section from the water bath and swift removal and dipping of tissue section into and out of processing reagents.

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Conflict of Interest

Nil.

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