



Study Of The Prevalence Of Various Artifacts In Histopathology

¹Dr. Shaista Choudhary, ²Dr. G Suba, ³Dr. Shilpa.S.J

¹Associate Professor, ²Assistant Professor, ³Post Graduate,

Department of Pathology, Dr.B.R. Ambedkar Medical College &Hospital, Bangalore, Karnataka, India

***Corresponding Author:**

Dr. Shaista Choudhary

Associate Professor, Department of Pathology, Dr.B.R. Ambedkar Medical College &Hospital,
Bangalore, Karnataka, India

Type of Publication: Original Research Paper

Conflicts of Interest: Nil

Abstract

Background :The accurate diagnosis of various lesions under microscope requires preparation of tissue sections, usually stained, that represents as closely as possible their structures in life. The preparation of high-quality sections requires skill and experience in the field of laboratory discipline. Most often, pathologists encounter slides that are either improperly fixed or mishandled during tissue processing, resulting in alterations in tissue details. Such changes are classified as “artifacts.” Artifact refers to “An artificial structure or tissue alteration on a prepared microscopic slide as a result of an extraneous factor.” They are the major source of diagnostic problem. The aim of this article is to review the various causes of artifacts and how to identify and prevent them from interfering in the accurate diagnosis of lesion

Aims And Objectives: To study the prevalence and patterns of various artifacts in histopathology
Materials & Methods :This was a prospective observational, quantitative study involving analysis of 500 consecutive histopathology slides in a duration of 6 months from March 2021 to August 2021. Slides were scanned for artifacts on a daily basis. These were the slides routinely stained with hematoxylin and eosin from the specimens sent to histopathology department at Dr.B.R.Ambedkar medical college and hospital, Bangalore. These slides were observed microscopically for artifacts encountered due to problems in tissue processing i.e from fixation to mounting of sections.

Results : The present study included 500 consecutive histopathology slides. Out of 500 slides observed 425 (85%) showed artifacts with varying intensity from mild to major artifacts.Many of the slides showed more than one pattern of artifacts and the most common artifact that was encountered during this study was Folding artifact (71%).

Conculsion : Folding artifacts were the most common artifact observed in this study. Skilled technicians, proper measures and vigilant care taken during tissue processing is needed in order to prevent/minimize the occurrence of artifacts and to improve accuracy of tissue diagnosis and avoid misinterpretation/ misdiagnosis due to artifacts.

Keywords: Artifact, diagnosis, histopathology, microtome, tissue specimen

Introduction

Histopathology refers to the microscopic examination of tissue to study the manifestations of diseases and it remains as a gold standard for diagnosing of various lesions.[1] The tissue sent for histopathological examination goes through the entire process starting

from tissue fixation till the sections are mounted on the glass slides.[1,2] This entire process requires skilled and experienced technical staff who has adequate knowledge and the main goal is to preserve the morphology of cells as close to how they were within the body before surgical removal.[1,3] The

various steps in slide preparation are fixation, tissue processing, embedding, microtomy, staining and mounting.[1] In spite of using Automated tissue processors nowadays for preparation of sections and staining, many artifacts are still encountered which may interfere with diagnosis by creating confusion and lead to an incorrect or inconclusive interpretation.[1,3] An artifact can be defined as an artificial structure or tissue alteration on a prepared microscopic slide as a result of various external factors.[3] Accurate diagnosis of the lesions under the microscope requires high quality sections and error at any stage of tissue processing can cause artefacts.[1] Artifacts can occur at any stage of tissue processing in slide preparations so it is important to know the various artefacts.[1] Some commonly occurring artifacts are folding of sections, split sections, fixation artifacts, dry mounting, air bubbles, excess mountant, dust particles, stain mucks, scoring artifacts, floaters, overstaining/light staining of slides etc.[1,3,4]

The present study was done to know the prevalence of artifacts that occur in the slides because of errors during tissue processing and list the various types of artifacts encountered. So based on the type of artifacts, we can find the cause and undertake necessary preventive measures to avoid them.[1,5]

Materials And Method

This was a prospective observational, quantitative study involving analysis of 500 consecutive histopathology slides in a duration of 6 months from March 2021 to August 2021. Slides were scanned for artifacts on a daily basis. These were the slides

routinely stained with hematoxylin and eosin from the specimens sent to histopathology department at Dr.B.R.Ambedkar medical college and hospital, Bangalore. These slides were observed microscopically for artifacts encountered due to problems in tissue processing i.e from fixation to mounting of sections. Patient’s personnel details like name, address and images of patients’s identity were not used in this study. Approval from Institution Ethics Committee (IEC) was obtained before starting this study.

Inclusion Criteria: All histopathology slides that were reported during the duration of the study.

Exclusion Criteria: Inadequate material

Results And Observation

The present study included 500 consecutive histopathology slides. Out of 500 slides observed 425 (85%) showed artifacts with varying intensity from mild to major artifacts.

The different artifacts that were observed during this study were split section (Fig.1),folding artifacts (Fig.2), scoring artifact (Fig.3), tissue tear (Fig.4), parched earth appearance (Fig.5), stain mucks(Fig.7), dust particles, overstaining (Fig.6), light staining, air bubbles(Fig.8), floater and thick sections. The results are tabulated below in Table.1.

Many of the slides showed more than one pattern of artifacts and the most common artifact that was encountered during this study was Folding artifact (71%)

Table 1: Types of artifacts expressed in numericals and percentage

Types of artifacts (n=425)	Number of slides showing the artifact	Percentage of slides showing artifacts
Folding artifact	302	71%
Tissue tear	224	52%
Split section	36	8.4%
Excess mountant	10	2.3%
Stain mucks	83	19.5%
Dust particles	27	6.3%

Overstaining	17	4%
Lightly stained	22	5.1%
Air bubbles	6	1.4%
Scoring	20	4.7%
Floater	2	0.4%
Thick sections	8	1.8%

Fig 1: Split section

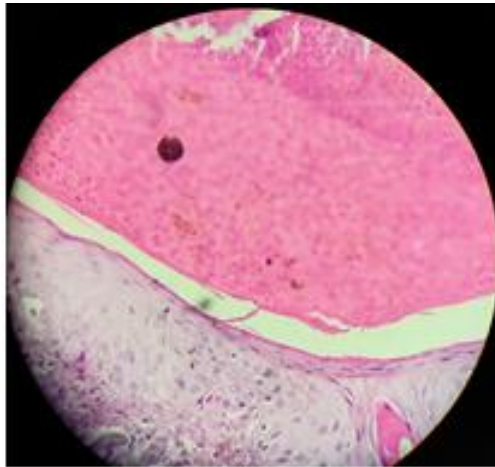


Fig 2: Folding artifact

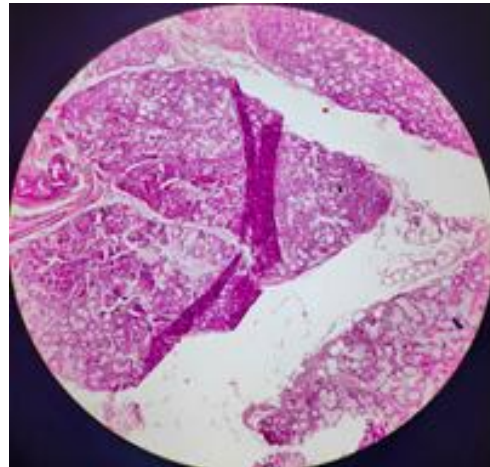


Fig 3: Scoring artifact

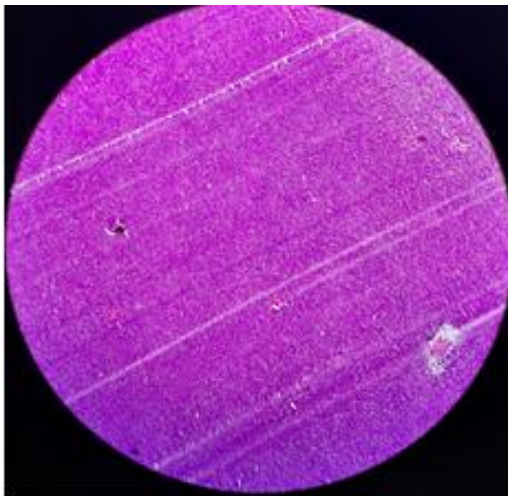


Fig 4: Tissue tear

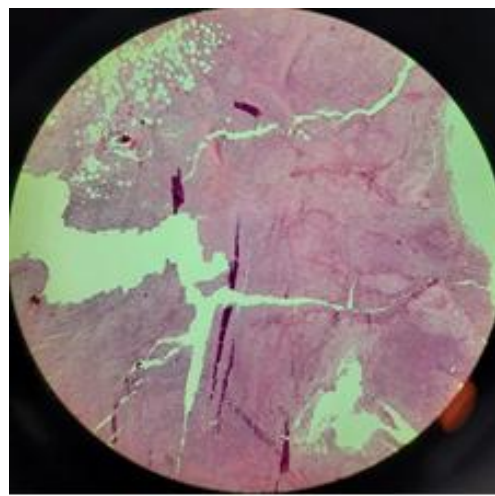


Fig 5: Parched earth appearance

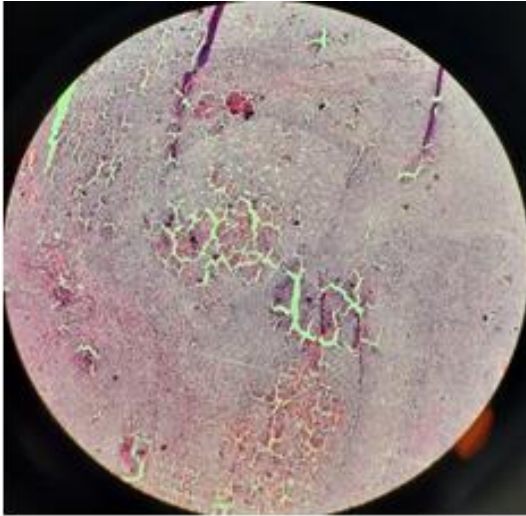


Fig 6: Overstained slide

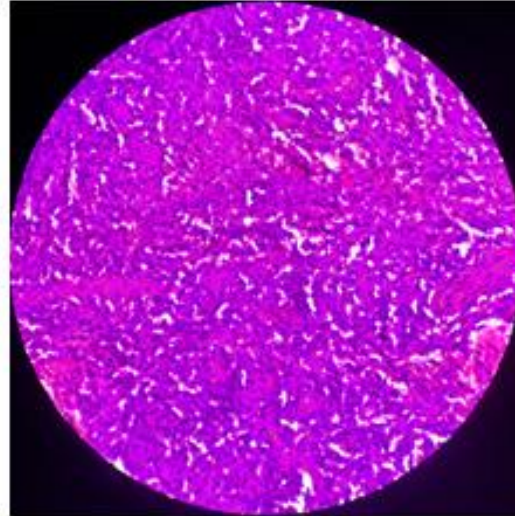


Fig 7: Stain mucks

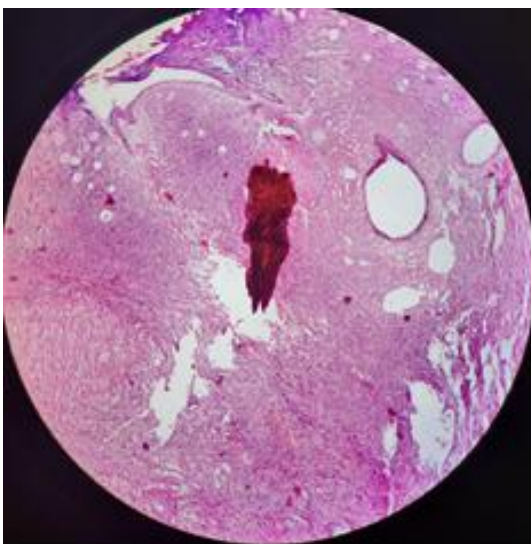
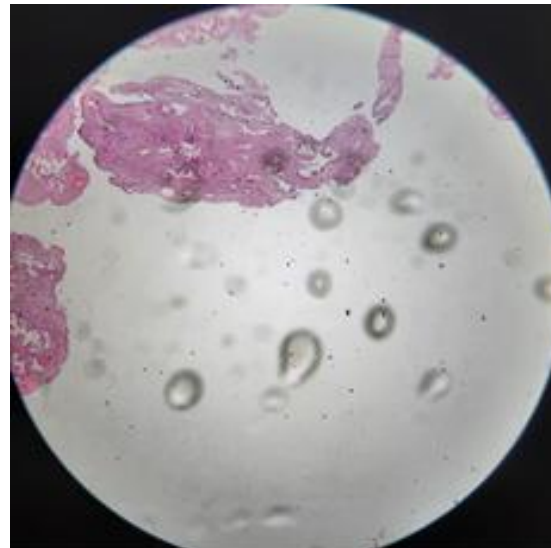


Fig 8: Air bubbles



Discussion

The toughest challenge is to recognize the artifacts and take appropriate measures to avoid it. Although fixation is necessary to maintain the tissue components and prevent decomposition, it can itself be a major cause of artifact. If the procedure is not carried out under optimal conditions, if fixative does not penetrate into the tissues due to lack of access, big specimen or because of the nature and quality of the particular reagent used, artifact can occur.[4,5] This can be prevented by using adequate concentration of fixative and keeping the specimen in it for an adequate time. Tissue folding artifacts occur

during lifting of tissue sections and was the most common artifact that we encountered in our study. Tissue tear is produced when the tissue adheres to the undersurface of the blade. They can be avoided by transferring sections to new water bath or by passing light of Bunsen burner over the section. Adding small amount of detergent to it is also helpful.[1,2] Increase temperature of water bath can cause expansion of tissue beyond its limit and causes “Parched Earth (crackes)” appearance and cool water bath causes excessive wrinkling of tissue. Both of these can be prevented by maintaining an optimal temperature.[6] Formation of air bubbles under the coverslip was less common. Using inadequate

mounting medium or very thin mountant causes it. Insufficient or excess mountant, positioning of the coverslip, use of small coverslips, contamination of slides with dust particles or pollens are other artefacts that can be encountered.[1] Usage of adequate amount of mountant with proper consistency and correct mounting technique can avoid this. Tiny air bubbles trapped during mounting should be removed carefully by applying mild pressure using a blunt needle.[1,3] Floaters are small pieces of unrelated tissue in the slides. They appear either during grossing, processing or floatation of tissue sections. This artifact can be avoided if only one specimen is grossed at a time. The cutting board and scalpel should be cleaned thoroughly after grossing each specimen and water in the water bath has to be changed frequently.[1,8] According to a study conducted by Mahesh S. et al. folding artifacts were the most prevalent pattern consistent with our study and they concluded that proper technical measures need to be employed in order to prevent/minimize the occurrence of artifacts in a skillful manner, as they may pose diagnostic difficulties.[1] A review article by Ekundina VO et al. focused on identifying artifacts, their potential cause and remedies so that misinterpretation and difficulty in diagnosis can be overcome and help microscopist to come into definite diagnosis just like the intent of our study.[2]

Conclusion

Folding artifacts were the most common artifact observed in this study. Skilled technicians, proper

measures and vigilant care taken during tissue processing is needed in order to prevent/minimize the occurrence of artifacts and to improve accuracy of tissue diagnosis and avoid misinterpretation/misdiagnosis due to artifacts.

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