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Artifacts in Histopatholohy – A Microscopic View

E.P. Sridevi Anjuga, N. Aravindha Babu[2], K.M.K.Masthan[3],

[1]Post Graduate Student, [2]Professor, [3]Professor and Head, Department of Oral Pathology and Microbiology, [4]Reader, Department of Oral Pathology and Microbiology, Sree Balaji Dental College and Hospital, Bharath Institute of Higher Education and Research, Pallikaranai, Chennai

Abstract

Histopathological examination is considered a gold standard procedure for reaching a final diagnosis of various human body lesions. However, it is limited by a number of changes in normal morphological and cytological characteristics that occur as a result of presence of artifacts. The purpose of preparing a biological specimen for microscopic studies is to obtain sufficient and reliable diagnostic knowledge which is a true representation of the specimen, whether it is for study or diagnosis. These objects may occur during procedures for surgical removal, fixation, tissue processing, embedding, and microtomy, and staining and mounting.They contribute to misinterpretation of the diagnosis of histopathology but often they bring the limelight into the diagnosis. This article discusses the common artifacts which we commonly encountered during slide examination

Keywords: *Artifacts, Histopathology, Tissue processing, Specimen*

Introduction

Histopathology is a science totally based on microscopic analysis and examination. The basic criteria for making a definitive diagnosis include the proper biopsy procedure, proper techniques for fixation and processing, sufficient sectioning and staining[1]. Sometimes, however, the existence of a foreign material or tissue alteration can cause uncertainty and lead to an incorrect or inconclusive interpretation[1]. Artifact can be described as artificial unrelated, self-colored features found in tissues sections[2]. They occur in tissue sections prior to fixation, during fixation, specimen grossing, tissue processing, sectioning, staining and tissue preservation[2]. Artifacts can result in alteration of normal morphological and cytological features, or even lead to complete tissue uselessness[2]. In some situations an artefact may compromise a precise diagnosis[2]. Knowing and understanding about artefacts is important because learning to recognise them can prevent misdiagnosis[2].

CAUSES OF ARTIFACTS[1]:

1. Clinical application of chemicals
2. Local injection of anesthetics
3. Surgical suctioning
4. Excessive heat
5. Freezing
6. Surgical mismanagement of specimen
7. Insufficient tissue fixation
8. Improper fixation medium
9. Incorrect tissue processing
10. Embedded sponges
11. Improper staining.

According to the stage at which they are formed artifacts can be classified into different categories as artifacts produced during[3,4,5,6,7]:

1. During surgery

a) Injection artifacts

b) Forceps artifacts

c) Crush/squeeze artifacts

d) Fulguration artefacts

e) Split artifacts.

f) Starch artifacts

g) Sutural artifacts

h) Autolytic artifacts

2. During fixation

a) Prolonged fixation

b) Improper fixation

c) Delayed fixation

d) Shrinkage artifacts

e) Formalin pigments

f) Mercuric pigments

g) Osmolality of fixative solution

h) Ice crystal artifact

i) Freezing artifacts

j) Microwave fixation artifact

3. Artifacts related to bone tissue

a) Bone dust artifact

b) Over decalcification

c) Incomplete decalcification

4. Artifacts during processing and embedding

a) Dehydration

b) Orientation artifact

c) Loss of soluble substance

5. Artifacts related to microtomy

a) Cutting artifacts

b) Moth eaten effect

6. Artifact related to floatation and mounting:

a) Prolonged floating

b) Floater artifact

c) Folds and wrinkles

d) Contamination

7. Staining artifacts

8. Microscopy artifacts

DURING SURGERY:

INJECTION ARTIFACT:

• Insertion of a needle may result in haemorrhage with extravasation that masks cellular architecture[1].

• The connective tissue bands can be separated by vacuolization[1].

FORCEPS ARTIFACT:

• The surface epithelium may be forced to form small 'pseudocysts' through the connective tissue[1].

• Compression of specimen leads to loss of cytological details. Nuclear dimensions are especially affected[1].

CRUSH / SQUEEZE ARTIFACT:

• Crushing produces a destructive and dangerous type of artefact that reshapes tissue morphology and squeezes chromatin from nuclei[1].Microscopically, the cellular features are not recognizable and nuclei appear darkly stained and distorted[3] .These are usually seen at the periphery of the lesion[4].

FULGURATION ARTIFACT:

• Epithelial cells may appear disconnected, with the nuclei assuming a spindle, pallisading configuration and the epithelium is separated from the basement membrane[2].

• The fibrous connective tissue, the fat and the muscle can appear opaque and amorphous[3].

• The epithelium and the connective tissue exhibit an amorphous appearance due to protein coagulation[3].

SPLIT ARTIFACTS:

· This artefact can lead to a split between epithelium and connective tissue, resulting in a false impression of vesiculo – bullous lesions[5].

STARCH ARTIFACTS:

· These appear as numerous blue, small, spherical structures in stained sections of hematoxylin and eosin. In oral cytosmear, they appear 5–20 μm in diameter as refractile, glassy, polygonal bodies, with a central dot or Y-shaped structure which may be misinterpreted as a pyknotic nucleus or for cells with mitosis[3,6]. This could also be identical to epithelial cells[3,6].

SUTURAL ARTIFACTS:

• May consist of isolated fragments or complete fibre-bundles cut into transverse, oblique or longitudinal planes[2,7]. Sometimes description of the fibre structure can be seen after careful inspection of H&E-stained sections and these silk sutures show a clear birefringence under polarised light and this can be useful in their identification[2,7].

• Silicone gel is used to stop bleeding often. The presence of foam gel in the histological section can create blood-filled distorted space surrounded by a slightly basophilic gelatin wall[6].

• There may be no pathological significance to the presence of a suture in the histological specimen[2,7].

DURING FIXATION AND TRANSPORT:

PROLONGED FIXATION :

· Prolonged fixation may lead to secondary shrinkage and hardening, resulting in tissue separation, giving an appearance of empty spaces[5].

IMPROPER FIXATION:

· Improper fixation results in autoysis. Autolyzed tissue displays increased eosinophilia due to the loss in cytoplasm of normal basophilia imparted by RNA, and increased binding of eosin to denatured intracytoplasmic proteins and cytoplasm vacuolization. Nuclear changes include pyknosis, karyolysis and karryohexis[3].

DELAYED FIXATION:

· Delayed fixation induces changes in the form of cytoplasmic clustering and cell shrinkage. It is difficult to separate the nuclear chromatin, and the nucleoli are also not visualised[5].

· Vascular structures, nerves and glands show the lack of detail, and an impression of scarring or loss of cellularity is seen[5].

SHRINKAGE ARTIFACTS:

• These artifacts are seen as a change in volume of tissues[5]. This is due to cellular respiration inhibition as well as changes in membrane permeability[5,7]. As a result, tissues attached in life may be separated from one another, leaving empty spaces5,[7].

FORMALIN PIGMENTS:

• Formaldehyde has a normal oxidising tendency, producing formic acid. Heme from red blood cells and formalin bind together to form a formalin – heme complex that occurs in parts of tissue as brown black amorphous to microcrystalline granules[3].

MERCURIC PIGMENTS:

• Usually mercury chloride containing a fixative creates dark brown granular deposits. The pigment to mercury chloride is extracellular[3].

OSMOLALITY OF FIXATIVE SOLUTION:

• Hypertonic fixative solutions cause cell shrinkage and increased extracellular spaces while isotonic (300–320 mOsm) and hypotonic fixatives may contribute to swelling of the cells[3]. Usage of a slightly hypertonic solution (400–450 mOsm) obtained the best results[3].

ICE CRYSTAL ARTIFACT:

• It appears as intercellular clefts in highly vascular tissue, and skeletal muscle intracellular clefts and vacuoles[3].

FREEZING ARTIFACT:

· Appear as Swiss cheese holes in epithelium (Interstitial vacuoles along with vacuoles inside the cell cytoplasm) and tissue spaces[3]. Sometimes, a granular paranuclear condensation of cytoplasm produced by dehydration of the cells as a result of freezing combined with fixation process is seen[1].

MICROWAVE FIXATION ARTIFACT: Underheating results in poor sectioning quality, while overheating produces vacuolation,

overstained cytoplasm and pyknotic nuclei[3].

ARTIFACTS RELATED TO BONE TISSUE:

BONE DUST ARTIFACTS:

· Undecalcified resin sections of bone when cut, dust is produced[3]. Some of these fragments are deposited on the cut surfaces while others may be implanted more deeply in the specimen. In hematoxylin and eosin-stained sections, it stains strongly with hematoxylin[3]. When deposited in bone marrow, it stains black with von kossa, indicating its origin from calcified trabecular matrix[3].

OVER DECALCIFICATION:

· Overdecalcified sections stain strongly with eosin and show a marked loss of nuclear basophilia. Nuclear and cytoplasmic features is poorly preserved[3].

INCOMPLETE DECALCIFICATION:

· Bony trabeculae stain strongly with hematoxylin and the adjacent soft tissue is severely disrupted[3].

ARTIFACTS DURING PROCESSING AND EMBEDDING:

DEHYDRATION:

• Excessive dehydration will give a dry homogeneous appearance to the tissue. This may also cause tissue sections to crack and become heavily stained[4].

• Insufficient dehydration results clogging of tissue and ineffective staining or opacity inside section[4].

ORIENTATION ARTIFACTS:

• Improper orientation in the slide can lead to disorderly organised histological features. For skin orientation, it must be placed in such a way that the epithelial margins, subcutaneous tissue and deeper layers are all flat to the bottom so that the finished slide displays all strata[3].

• Exposure of the specimen for too long during embedding findings indicates loss of soluble substances when neutral fat is removed from fat cells , leaving normal ovoid spaces[5].

LOSS OF SOLUBLE SUBSTANCES:

• The preparation of paraffin wax, cellulose nitrate and most embedded parts of synthetic resin requires the use of fat solvent[3]. Thus, during the processing of adipose tissue, the fat can separate from fat cells and appear as ovoid spaces enclosed by a cytoplasm surface.

For example: lipoma and cholesterol clefts in odontogenic cysts[3].·

ARTIFACTS RELATED TO MICROTOMY:

CUTTING ARTIFACTS:

• Split line in tissue segment in microtome knife due to nick[6].

• Tissue compression due to blend microtome knife[6].

• Chatter artefact due to knife edge vibrations, knife or block holder loosening and extreme steep clearance angle[6].

• If the tissue is tangentially cut, the connective tissue cores can become entangled in the epithelium, giving a false impression of invasive squamous cell carcinoma[3].

MOTH EATEN- EFFECT:

· It occurs with a greater thickness due to excessively rough trimming of the paraffin blocks.

· This brings out the tissue fragments from the block face and these appear in subsequent thin sections as void spaces or holes with their long axis parallel to the edge of the knife[3].

ARTIFACTS RELATED TO FLOATION AND MOUNTING:

PROLONGED FLOATING:

· Tissues can expand beyond their actual size and become distorted and give epithelium an acantholytic appearance mimicking edema[3].

FLOATER ARTIFACT:

· Floaters are bits of tissue that were seen on slides not belonging to them[3]. They may have floated during processing and may be the result of sloppy bench cutting procedures using dirty towel, knife, gloves and improper water bath cleaning[3]. These can have tissues which are transported to the next case[3].

FOLDS AND WRINKLES IN THE SECTION:

· These artefacts look like darker stained strands. Any residual wrinkles and fords in water bath portion can be eliminated by stretching with forcep and gentle tapping. Overstretching can cause tear in the tissue section during these procedures resulting in an acantholytic appearance in epithelium[3].

CONTAMINATION:

• Often water baths may become contaminated with dust, hair, previous section residual cells, etc. Contamination with squamous epithelial cells and keratinous debris has been shown to be caused by fingers to remove air bubbles or sneezes or cough[3].

• Higher water bath temperature can cause tissue expansion beyond its limit and shows dark pyknotic nuclei or nuclear bubbling called "heating artifact"[6].

• Contamination by sneezing and coughing caused by exfoliated squamous cells may also cause artefacts[6]. Artifact due to tissue presence, which does not belong to the section, is due to unclean bath of water. Slide adhesive thick coating will take over the stain and result in poor quality section[6].

STAINING ARTIFACTS:

• Failure to extract wax from parts completely results in staining deficiency known as residual wax artefact[4].

• The presence of residual wax can lead to insufficient stain penetration leading to a stainless area and also to subtle effects on nuclear staining leading to muddy nuclei appearance, lacking in detail[6].

• The appearance of external or internal dust particles on the slide results in artifactural alterations[6].

MICROSCOPY ARTIFACTS:

Impurities of the dust particles that may be present on the slide internally or externally may bring about artifactual changes. Fatty films are found resulting in a foggy appearance due to uncleaned lenses or greasy deposits on ocular pieces due to eyelashes[7].

Conclusion

Artifacts are identified in most microscopic sections, and play a role in histopathological diagnosis interpretation. Most of the microscopic section shows artefacts, which play a significant role in diagnostic misinterpretation. Although the existence of artefact in the diagnosis provides some clue, most of the artefact is accidental and causes diagnostic pitfall. The artefact must also be established and resolved such that misinterpretation and diagnostic difficulties can be addressed for a definitive diagnosis.

Ethical Clearance – Not required since it is a review article

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