Review Article

Artefact & Classification

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Abstract: Literal meaning of artefact given by “Oxford Advanced Learner Dictionary” is a “thing made by the people”. In medical science the “fact” is not true; but we observe routinely is called artefact. These defects are referred to as artifacts. They lead to misinterpretations of histopathological diagnosis. This paper attempts to put all various types of artifacts that are observed in histopathology.

Keywords: Oxford Advanced Learner Dictionary, artifact

INTRODUCTION

An artefact (L. ars + factum-made) in histology means any non-natural feature or structure accidentally introduced into something being observed or studied [1]. According to Bernstein, Artefact refers to “An artificial structure or any tissue alteration on a prepared microscopic slide produced by some extraneous factors” [2]. Tissue artefacts are a recognized, all-too-common occurrence that are encountered in all surgical pathology practices [3]. Artefacts are defects or tissue distortions that may occur as a result of the way the tissue has been handled, right from the time of biopsy surgically obtained till the entire histopathological procedures are performed on it. Although many of these artefacts may obscure a definitive diagnosis of the tissue pathology, most are duly recognized by the experienced pathologist [4]. Artefacts which occur during the processing of small biopsy specimens can cause sufficient tissue distortion to impair interpretation and can be a considerable source of nuisance[4]. Some artefacts are easily distinguishable from normal or diseased tissue components and some are difficult to distinguish from such entities. In histological and cytological terms an artefact can be defined as a structure that is not normally present in the living tissue [5].

Examination of microscopic sections of animal tissues reveals facts which are not always related to its normal histology or pathology. Processing of tissue specimens consists of lengthy procedures from the stage of surgical removal to the stained and mounted microscopic sections. Defects are common in tissue sections as a result of faulty procedures. These defects are referred to as artefacts [2]. Artefacts result in alteration of normal morphological and cytological features or they may even lead to complete uselessness of the tissue, thus creating serious errors and misdiagnosis of correct histopathological impression. Artefacts may occur during surgical removal, fixation, processing, embedding, microtomy and staining procedures [5].

The aim of a good histopathological technique is to produce microscopic preparation of tissues, usually stained, that represents as closely as possible, their structures in life. But this is not always possible and some sort of tissue morphology is bound to be seen [6].

Just like: “All photos are accurate. None of them is the truth” - Richard Avedon

Biopsy specimens of oral cavity are of small size and fine texture resulting into higher effectiveness of artefacts on them. Some artefacts are easily distinguished from normal or pathological tissue components, and some are difficult to distinguish from such entities [5].

Inadequate biopsy specimens may be encountered somewhat frequently by the pathologist. Tissue artefact incurred at surgery, inappropriate sampling of the tissue for the type of pathologic condition suspected, or an insufficient clinical history-among other deficiencies- may hamper the pathologist in his interpretation [7].
Availability of modern technique nowadays have minimize the occurrence of artefacts. These techniques are costlier than the conventional techniques but as said: “Good wine ruins the purse; bad wine ruins the stomach” – Plato.

The problem is recognizing artefacts as such when they do occur and not confusing them with normal tissue components or pathological changes. In some situations the presence of an artifact can compromise an accurate diagnosis and can create great amount of confusion in making a diagnosis for the histopathologists [5].

It is important to have thorough knowledge on artefacts, so that appropriate precautionary measures can be taken to avoid or minimize their occurrence. The ability of oral pathologists in interpreting a biopsy correctly is directly proportional not only to the quality, but also to the quantity of the specimen [8]. It is important to know and understand about artefacts as by learning to recognize them, so that we can avoid misdiagnosis [5].

“Your eyes can’t see what your mind does not know” - Socrates

So to identify any artefact, the competent histopathologist must possess a perfect eye and mind co-ordination to differentiate between normal/pathological finding and an artefact.

CLASSIFICATION OF ARTEFACTS
A. According to Kumar K, Shetty DC, artefacts are classified as;
   1. Artefacts incurred during oral mucosal biopsy procedures
      Type of biopsy Artefacts
      a. Incisional biopsy
          Squeeze artefacts.
          Curling artefacts.
          Artefacts due to injection of local anesthetic solution into the lesion (vacuolations).
          Starch artefacts.
          Foreign bodies causing artefacts.
      b. Excisional biopsy
          When the tissue is removed with excessive force, the epithelium and connective component may suffer important damage.
      c. Forceps and scalpels
          Forceps used to grasp the specimen may perforate the latter, leaving gaps and creating compression zones around the tissue.
      d. Electroscalpel and CO2 laser scalpels, cauterizes the vessels, causing no bleeding, induction of thermal damage
          The heat generated by an electroscalpel gives rise to alterations, such as tissue protein coagulation –resulting in an amorphous epithelial and connective tissue appearance. In such situations, the epithelial cells become fusiform and hyperchromatic.

2. Artefacts incurred during various fixative and tissue processing procedures
   Procedures Artefacts
   a. Fixation: Fixation for light microscopic preparations
       Shrinkage artefacts.
       Pigmentation artefacts-tissues are fixed in solutions containing formalin or mercury.
       Streaming artefacts.
       Diffusion artefacts.
       Artefacts due to chemical changes (changes in the form of cell shrinkage and cytoplasmic clustering).
       Acetone or 70% alcohol sharply dehydrates the tissues, complicating epithelial staining and poorly fixing the connective tissue elements.
   b. Fixation for electron microscopic preparations
   c. Freezing of the tissue
       Ice crystal artefacts.
       Disruption artefacts.
   d. Tissue processing
       Reagents: contamination, pH, concentration, temperature, timing, shrinkage artefacts, vacuolization of the specimen.
   e. Embedding
       Tearing artefacts and holes.
   f. Tissue sectioning
       Wrinkling, curling, nicks, alternate thick and thin sections, chaffer, wrinkling and folding, floaters.
   g. Staining
       Hydration error, reagents, light staining, dark stain, improper contrast, fading stain, blotching.

B. According to Bindhu PR, Krishnapillai R, the stage at which artefacts are formed they can be classified into different categories as artefacts produced during:
   a. Surgical biopsy procedure.
   b. Fixation.
   c. Tissue processing.
   d. Embedding.
   e. Microtomy.
f. Mounting.
g. Staining.
h. Cover-slipping.

C. According to Rastogi V, Puri N, artefacts are classified as:
1. Artefacts during oral biopsy procedures
   a. Due to delay in fixation.
   b. Forceps artefacts.
   c. Curling artefacts.
   d. Squeeze artefacts.
   e. Compression artefacts.
   f. Haemorrhage artefacts.
   g. Split artefacts.
h. Artefacts due to inking during surface preparation.
   i. Artefacts due to cotton and starch.
   j. Fulgeration or heat artefacts.

2. Fixation artefacts
   a. Shrinkage and hardening due to prolonged fixation in formalin.
   b. Artefacts due to delayed fixation.
   c. Artefacts due to freezing during transport before fixation.
   d. Pigmentation artefacts.
   e. Streaming artefacts.
   f. Artefacts due to false localization of extraneous material.
   g. Diffusion artefacts.
h. Artefacts due to chemical changes.
   i. Artefacts due to overheating during microwave fixation.
   j. Crush artefacts.
k. Ice crystal artefacts.

3. Artefacts during decalcification.

4. Artefacts during tissue processing
   a. Shrinkage artefact during dehydration in too great concentration of alcohol.
   b. Crumbling and crystallization of tissues due to prolonged immersion in clearing agents.

5. Artefacts during embedding
   a. Artefacts due to incorrect orientation of tissues and due to prolonged exposure during embedding.
   b. Tearing artefacts and holes due to insufficient dehydration prior to clearing.

6. Artefacts during microtomy
   a. Wrinkling, curling, nicks in tissue, alternate thick and thin sections are some of the artefacts.
   b. Folding of tissue sections.
   c. Chatters or chaffers.

7. Artefacts during staining
   a. Artefacts in terms of altered intensity and nature of staining, due to old, decomposed dyes, impurities present in the dye, leaching of certain substances from tissues into the dye.
   b. Patches or blotchiness on slide due to improper clearing of the wax.

8. Artefacts during mounting
   a. Artefacts in the form of bubbles under the cover slip.

9. Miscellaneous artefacts
   a. Bone artefacts during sawing, drilling and decalcification procedures.
   b. Reprecipitation artefacts due to lack of agitation and inadequate volume of decalcifying fluid.
   c. Artefacts of undecalcified bone consists of cracking of matrix in resin sections.

D. According to Khan S, Tijare M, artefacts are classified as:
1. Pre-fixation artefacts
   a. Artefacts due to surface preparation, tattoo pigment artefact.
   b. Surgical artefacts.
      ❖ Forceps artefacts.
      ❖ Crush or squeeze artefacts.
      ❖ Fulgeration artefacts.
      ❖ Injection artefact.
      ❖ Sutural artefact.
      ❖ Artefacts due to contamination.

2. Fixation artefacts
   a. Pigment artefacts.
   b. Shrinkage artefacts.
   c. Streaming artefacts.
   d. Diffusion artefacts.
   e. Artefacts due to microwave fixation.
   f. Artefacts during freeze-drying-ice crystal artefacts.
   g. Artefacts due to prolonged fixation.

3. Processing artefacts
   a. Artefacts during post fixation treatment.
   b. Artefacts during dehydration.
   c. Artefacts during clearing.
   d. Artefacts during impregnation.
   e. Artefacts during embedding.
   f. Artefacts due to poor processing.

4. Cutting artefacts
   a. Artefacts related to microtomy:
      ❖ Thick and thin sections.
      ❖ Chatter artefacts.
      ❖ Splitting of sections at right angle.
      ❖ Knife lines.
      ❖ Venetian blind effects.
      ❖ Excessive compression.
      ❖ Incomplete sections.
   b. Artefacts related to tissue floating water bath.
   c. Artefacts related to oven/hot plate.
   d. Artefacts during lifting of tissue sections.
   e. Artefacts due to section adhesive and mounting.
5. Staining artefacts
a. Artefacts due to residual wax.
b. Artefacts due to contaminated staining solution.
c. Artefacts due to stain deposits.
d. Artefacts due to incomplete or unstained areas.

6. Mounting artefacts
a. Artefacts due to air bubble entrapment.
b. Residual water.
c. Excessive use of mounting media.

7. Microscopy artefacts
a. Due to dust particle impurities.
b. Fatty films or foggy appearance due to uncleaned lenses or greasy deposits on eyepieces.

**Working Classification**
Artefacts are classified as follows:

1. Prefixation artefacts
I. Artefacts due to surface preparation.
II. Tattoo pigment artefacts.
III. Post mortem artefacts.
IV. Surgical artefacts:
   - Artefacts due to contamination.
   - Injection artefacts.
   - Forcep/Crush artefacts.
   - Fulgeration/Heat artefacts.
   - Artefacts due to haemostatic agents.
   - Compression artefacts.
   - Split artefacts.
   - Curling artefacts.
   - Sutural artefacts.

2. Fixation artefacts
i. Artefacts due to delayed fixation.
ii. Artefacts due to prolonged fixation.
iii. Artefacts due to false localization of externous material.
iv. Artefacts due to chemical changes.
v. Diffusion artefacts.
vi. Artefacts during freezing-drying ice crystal artefacts.
vii. Artefacts due to microwave fixation.
viii. Zonal fixation artefacts.
ix. Streaming artefacts.
x. Artefacts due to incomplete fixation.
xii. Pigment artefacts.

3. Processing artefacts
i. Artefacts during post fixation treatment.
ii. Artefacts during dehydration.
iii. Artefacts during clearing.
iv. Artefacts during impregnation.
v. Artefacts during embedding.
vi. Artefacts due to poor processing.

4. Tissue sectioning and Mounting artifact
i. Knife lines.
ii. Displacement of components.
iii. Nuclear meltdown.
iv. Drying artefacts.
v. Overexpanding sections.
vi. Water in section.
vii. Section too thick.
viii. Alternate thick and thin sections.
ix. Venetian blind effects.
x. Wrinkling and folding in tissue.
xi. Floater artefacts.
xii. Holes from rough trimming.
xiii. Draining before drying.
xiv. Excessive compression.
xv. Disruption.
xvi. Frozen section chatter.
xvii. Chaffer artefacts.
xviii. Coarse chatter.
xix. Contamination of mounted sections.
xx. Artefacts due to air bubble entrapment.
xxi. Tide mark due to adhesive.
xxii. Section not flat (poor adherence).

5. Staining artefacts
i. Residual wax.
ii. Mucus contamination.
iii. Contaminated staining solution.
iv. Bleaching of stain.
v. Fuzzy staining.
vi. Blotching of sections.
vii. Stain deposit.
viii. Incomplete staining.
ix. Unstained area in a section.

6. Microscopy artefacts

7. Miscellaneous artefacts
i. Bone artefacts during sawing, drilling and decalcification procedures.
ii. Reprecipitation artefacts due to lack of agitation and inadequate volume of decalcifying fluid.
iii. Artefacts of undecalced bone consists of cracking of matrix in resin sections.

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