

## Artifact in Histological Section

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### ABSTRACT

Microscopic examination of histological section of the tissue does not always related to normal histology or pathology. Processing of tissue specimen is a lengthy procedure. Defects are common in this lengthy procedure and they are referred as Artifact. Accuracy of the histopathological diagnosis depends on eliminating or minimizing the artifact in histological section. These artifacts may occur during surgical removal, fixation, processing, embedding, microtomy and staining procedures. It is therefore important to identify the common occurring artifacts during interpretation of oral biopsies.

**Key words:** Histological, Fixation, Processing, Embedding, Microtomy.

### INTRODUCTION

An artifact is defined as any structure or feature in the histological section, which is not normally present in the normal tissue, and it may come from outside sources. Some artifact are easily distinguished and some are not .when it is present it may confuse with normal tissue or any pathological changes. Presence of artifact can lead to inaccurate diagnosis. Hence, it is important to know and understand the commonly occurring artifact, so that we can avoid misdiagnosis. Artifact can occurs at any stage, which can be classified as the following

- ˆ Prefixation
- ˆ Fixation artifact
- ˆ Processing artifact
- ˆ Cutting artifact
- ˆ Floating and mounting artifact
- ˆ Staining artifact
- ˆ Microscopy artifact
- ˆ Prefixation artifact

These types of artifact are produced before the tissue is being fixed. Some of the coloring agent is used for marking the biopsy site and for

appropriate orientation of the specimen margins. Some commonly used reagents are Indian blue , alcian blue or alcian green ,silver nitrate. Sometime tincture iodine is used as decontaminant for biopsy site. These colored solution are used carefully and this should be mentioned clearly because it may interfere tissue processing and staining procedure

Large amount of anesthetics solution injected into site can lead to hemorrhage, vacuolation of epithelium and it may separate the connective tissue from epithelium. It can be avoided by using block injection technique or injecting well away from the site.

Some of the commonly used surgical instrument for the biopsy can penetrate the specimen and it can cause voids or tears and compression of surface epithelium into the connective tissue . If electrocautry are used for coagulation can drive surface epithelium into the connective tissue thus producing a pseudocysts and loss of cytoplasmic and nuclear feature .Electrocautry in parotid surgery causes oncocyctic changes in acinar cell<sup>1</sup>.

Heat from the laser used for biopsy may lead to detachment of epithelial cells, detachment of epithelium from the basement membrane also induce carbonization, nuclear elongation and vacuolar degeneration<sup>2</sup>. This can be avoided by using knives and electrical point.

Presence of suture material in microscopic tissue is also common artifact which causes damage to the microtome knives and leads to tear in tissue section<sup>3</sup>. Sometime foam gel is used to control bleeding. The presence of foam gel in histological section can cause distorted space filled by blood surrounded by slightly basophilic gelatin wall<sup>3</sup>.

Starch powder present in the gloves lead to starch artifact. These starch granule can be misinterpreted with pyknotic nucleus. Reusable tissue cassette if not cleaned properly can carry the older tissue specimen fragment.

#### **Fixation artifact**

Fixation is a process which is necessary to prevent tissue from diffusion of soluble component there by prevent autolysis and putrefaction. Depends on the nature and quality of the fixative agent artifact can occurs. The volume of the fixative is 20 times more than that of the specimen (less than 6mm). normally 10% formalin is used as fixative. Prolonged fixation cause difficult in sectioning with microtome and also cause "bleaching artifact" which give empty space appearance. If saline is used in hypertonic condition, it may cause shrinkage or crenation where as hypotonic saline cause swelling of the cells. This can be corrected by using phosphate buffered saline (PBS)

Evaporation of formalin during fixation can cause intra epithelial cleft formation and acantholysis. Some fixative agent contain mercuric chloride and picric acid may cause brown black granule and yellow stain throughout the tissue section. Sometime tissue are not fixed properly which may localized to some other place to produce "streaming artifact" this is because of the loss of glycogen in glutaraldehyde fixation.

#### **Processing artifact**

Processing is the procedure of replacing

the water content in the tissue specimen with supporting medium, which provide enough rigidity for tissue sectioning without damaging it.

Incomplete dehydration leads to entrapment of water into the tissue specimen which cause inadequate staining or opacity in tissue section, which can be prevented by frequent changing the solution. Over dehydration causes the tissue brittle and hard which make tissue sectioning difficult and interfere with staining properties.[4] inadequate infiltration in paraffin causes wrinkle in tissue section.[5] inadequate removal of clearing agent from tissue may cause crumbling and crystallization of tissue while sectioning.

Entrapment of air is common finding while embedding which lead to blind artifact which appears as zone compressed tissue separated by open space. Improper orientation of tissue lead to difficult to study tissue microscopically.

#### **Cutting artifact**

Thick and thin section appears due to loosely attached microtome blade. Split line in tissue section due to nick in microtome knife [6]. compression of tissue due to blunt microtome knife. Chatter artifact due to vibration in knife edge, loose in knife or block holder and excessive steep clearance angle. Incomplete section is due to incorrectly embedded tissue or incomplete impregnation or superficial cut section.

#### **Floatation and mounting artifact**

Entrapment of air bubble is due to poor floatation technique which lead to inadequate adherence of tissue to the slide. Higher temperature of water bath can cause expansion of tissue more than its limit and shows dark pyknotic nuclei or nuclear bubbling called "heating artifact". Some contamination of tissue may also occur by air borne fibers, microorganism, cellulose fibers, and hair. Contamination due to exfoliated squamous cell by sneezing and coughing may also cause artifact. Artifact due to Presence of tissue, which does not belong to the section, is due to unclean water bath. Thick coating of slide adhesive will take the stain and result in poor quality section.

#### **Staining artifact**

Residual wax should be removed completely before staining, presence of residual wax can cause inadequate penetration of stain leads to the area devoid of stain and also cause subtle effect on nuclear staining lead to muddy appearance of nuclei with lack of detail<sup>7,8</sup>. This can be corrected by prolonged xylene treatment and re-staining procedure. Precipitated stain lead to deposition of section. Contamination of solution by microorganism, foreign particle or expired solution lead to deposition on section<sup>7</sup>. Drying up of tissue section between last xylene and cover slipping lead to entrapment of minute bubble over the nuclei lead to lack of visible detail<sup>9</sup> called corn flake artifact. Excessive use of the mounting media can cause crystallization and cracking. Prolonged exposure to light can cause bleaching of stain.

Presence of dust particle external or internal to the slide leads to artifactual changes.

Unclean microscope lenses or greasy deposit on eyepieces leads to foggy appearance.

### CONCLUSION

Most of the microscopic section show artifact, which play an important role in misinterpretation of diagnosis. Few artifacts provide useful clues for diagnosis such as cholesterol cleft which appears as needle-like spaces due to dissolution of lipids during processing in radicular cyst / periapical granuloma, lacunar cells in nodular sclerosis, melanin pigment fluorescence induced by formalin in amelanotic melanoma, formation of Max Joseph spaces due to basilar degeneration in lichen planus. Though presence of artifact gives some clue in diagnosis, most of the artifacts are unintentional and cause pitfalls in diagnosis. So it is necessary to identify and overcome the artifact so that misinterpretation and difficulty in diagnosis can be overcome for definite diagnosis.

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