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IMMUNOHISTOCHEMISTRY OF ODONTOGENIC CYSTS & TUMOURS – A REVIEW ARTICLE

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Abstract:
Odontogenic cysts and tumours are lesions that are derived from the tooth producing tissues or their remnants that remain entrapped either within the jaw bones or into the adjacent soft tissues. From a biological point of view, some of these lesions represent hamartomas with varying degrees of differentiation, while the rest are benign or malignant neoplasm with variable aggressiveness and potential to develop metastasis. Immunohistochemistry is the in situ detection of specific antigens in tissue sections by means of an immunologic reaction (based on antigen -antibody binding). Today, immunohistochemistry has applications in a wide variety of oral lesions including cysts and tumours of odontogenic origin. The aim of this review will be to focus on the various aspects of immunohistochemistry in odontogenic cysts and tumours including its advantages, disadvantages, and applications & recent advances.
keywords: immunohistochemistry, odontogenic cysts, odontogenic tumours, advantages, disadvantages.

INTRODUCTION:

Over the years pathologists have used many special techniques to confirm, complement and refine the information they were able to obtain with their old faithful armamentarium i.e formalin fixation, paraffin embedding, and haematoxylin and eosin staining. These techniques include special staining, tissue culture, electron microscopy, immunohistochemistry (IHC) and molecular biology methods. It could be fair to say that today no special technique has influenced the way pathology is practiced as profoundly as immunohistochemistry or has even come close to it.[1]

Immunohistochemistry is the in situ detection of specific antigens in tissue sections by means of an immunologic reaction (based on antigen -antibody binding).The antigen is visualized in the form of an insoluble coloured reagent product in target cells and tissues using a light microscope.[2] Today, immunohistochemistry has applications in a wide variety of oral lesions including cysts and tumours of odontogenic origin.

Over the years many different investigation modalities have been utilized in diagnosis of odontogenic cysts and tumours. In recent years, Magnetic Resonance Imaging (MRI) has been increasingly used to evaluate

cysts and tumours of oral and maxillofacial region. Diffusion weighted magnetic resonance imaging (DWMRI) provides an additional paradigm for characterizing oral lesions.[4]

The clinical practice shows that the conventional X-ray methods extra oral and intraoral ensure enough information concerning removal of the cyst when they are single and small in size. It is known that panoramic radiography has a limited value for evaluating the margins and extensions of the lesion. Computed tomography (CT) examination aids in delineating the extent of the lesion especially for précising the osteolytic changes in different levels of the bone plates in the cranial, caudal, vestibular and lingual position.[5]

Fine needle aspiration biopsy (FNAB) is a semiotechnique method frequently used to analyze cyst like lesion content. Cell Block is a histological technique that allows microscopic analysis of cystic material and can be stored for future use as in immunohistochemical assays.[6]

Today immunohistochemistry is a powerful tool which over the time has helped us in gaining indispensible knowledge about the molecular biology of odontogenic cysts and tumours.[7] Oral pathologists investigate different aspects related to the molecular biology of cell populations, in an attempt to elucidate facts on origin, proliferative growth potential and invasion mechanisms by immunohistochemistry. Knowledge of the biologic behavior of pathologic entities of odontogenic origin obtained by IHC is essential for rendering the most appropriate therapeutic approach and establishing a prognosis for each case.

DISCUSSION:

Immunohistochemistry of odontogenic cysts and tumours encompasses a novel diagnostic modality in recent times. This discussion attempts to highlight the topic under following headings:

1) Immunohistochemistry techniques.
2) Odontogenic tumour markers.
3) Advantages and disadvantages of immunohistochemistry
4) Applications of immunohistochemistry
5) Recent Advances in Immunohistochemistry

1)IMMUNOHISTOCHEMISTRY TECHNIQUES:

Immunohistochemical techniques are used to identify substances in tissue by marking them with specific antibodies. The methods of labeling are immunofluorescence using fluorescent pigments and an immunoenzyme method.[69]

IMMUNOFLUORESCENCE (IF):

Immunofluorescence (IF) is an immunological staining method that uses antibodies labeled with fluorescent material such as fluorescein isothiocyanate (FITC) to detect the molecule connected to the antigen. It is used to detect antigens, antibodies, complements and causative factors of diseased tissues and to examine serologic reactions. IF techniques are classified by the labelled antibody reactions into direct fluorescent antibody test, indirect fluorescent antibody test and complement fixation test.[69]

DIRECT IF:

A fluorescent-labeled antibody is used to detect the antigen. When the labeled antibody detects the tissue, fluorescence appears at the site with the target substance, and that fluorescence can be observed by fluorescent microscopy. This type of test is used for detection of the autoantibody in vivo, mainly in autoimmune diseases such as lupus erythematosus, pemphigus vulgaris and bullous pemphigoid. Direct IF is also used for detection of pathogenic microbes in tissues.[69]

INDIRECT IF**:**

Indirect IF is a two-phase technique whereby an unlabeled primary antibody reacts against a target substance, and a labeled secondary antibody is reacted against the primary antibody. In bullous pemphigoid, for example, an IgG antibody circulating in the bloodstream reacts directly with the basal membrane of the patient. In indirect IF, a patient's serum is reacted against normal skin, and then a labelled anti-IgG antibody is reacted. If the IgG anti-basal membrane antibody is present in the serum, fluorescence is observed on the basal membrane zone.

COMPLEMENT IF:

An unlabeled primary antibody is reacted against the target substance and is provided with complement components. Then a labeled secondary antibody (labeled anti-C3 antibody) is reacted against the target substance.[69]

IMMUNOENZYME METHOD

In the immunoenzyme method, an enzyme rather than a fluorescent material is labeled with an antibody. Antigens, immunoglobulin and complements can be detected using the enzyme reaction. Enzymes such as peroxidise are labeled with an antibody to react against the target molecules in the tissue in the same way as in the IF technique. The presence of the target substance and its distribution are indicated by the

presence and distribution of the pigments. Immunoenzyme method has the following advantages over IF: the enzymatic reaction makes electron microscopic observation possible, the reaction is easy to observe, has a high detection range and the samples can be stored longer than those of IF.[69]

ELECTRON MICROSCOPY (EM) AND IMMUNO EM:

The electron microscope is a device that generates enlarged images of a specimen by emitting electron beams instead of visible light. Electron microscopy (EM) and immuno EM achieve magnifications of 1,000 times and greater, so they can be used to observe fine intracellular and intercellular structures (e.g., Birbeck granules in Langerhans cells) that are not visible by light microscopy. Immuno EM, a combination of immunostaining and electron microscopic observation, has greatly contributed to the development of dermatological science, such as in the identification of autoantigens in autoimmune blistering diseases. In scanning EM, electron beams reflected from an exposed object are detected. It is effective in revealing three-dimensional structure; however, the magnifying power is not as high as that of transmission EM.[69,20]

SUMMARY OF ODONTOGENIC TUMOR MARKERS:

Markers	**Clinical Significance**
CK 14,19	Differentiates odontogenic epithelial tumors from other epithelial tumors
Amelogenin	Expressed in odontogenic tumors with odontogenic epithelial component
Ameloblastin	Mutated in odontogenic tumors with odontogenic epithelial component
Nestin	Marker for odontogenic ectomesenchyme
Calretinin	Differentiates ameloblastoma from other tumors Differentiates unicystic ameloblastoma from

	odontogenic cysts
Bone Morphogenic Protein	Expressed in odontogenic tumors with dental hard tissue formation
Tenascin	Expressed in tumors forming calcified masses
HMGA2	Over expression in odontogenic mesenchymal tumors
Basement membrane proteins: Laminin 1	Marker for odontogenic epithelium

4)ADVANTAGES OF IMMUNOHISTOCHEMISTRY(IHC):[2]

- It is a highly sensitive and specific method.
- Formalin-fixation of tissues permits the convenient and safe handling of specimens containing human and animal pathogens.
- Both viable and non-viable organisms can be detected in routinely-fixed and processed tissues.
- It is an efficient means of detecting organisms which are slow or difficult to diagnose by viral isolation or bacterial culture e.g. *Mycobacterium* sp.
- Antigens can often be detected in autolyzed tissues.

DISADVANTAGES OF IMMUNOHISTOCHEMISTRY:[2]

- Even though immunohistochemistry is relatively a simple technique, it has some particularities and its outcome depends on many factors.

- Immunostaining methods require rigor of execution and may present significant bias. The block slices must preferentially present a thickness ranging between 3 and 7µm and must be deposited on slides previously prepared with some kind of adhesive.

- Slices less than 3µm thick could result in very weak immunostaining while those thicker than 7µm may lead to loss of tissue on the glass slide or hamper analysis of resultant immunostaining.

5)APPLICATIONS OF IMMUNOHISTOCHEMISTRY:[1,74]

PROGNOSTIC MARKERS IN CANCER

To predict the prognosis of tumours by identification of enzymes, tumour-specific antigens, oncogenes, tumour suppressor genes, and tumor cell proliferation markers. IHC is used for disease diagnosis, drug development, and biological research. Using specific tumour markers, physicians use IHC to diagnose a cancer as benign or malignant, determine the stage and grade of a tumour, and identify the cell type and origin of a metastasis to find the site of the primary tumour.

TUMOURS OF UNCERTAIN HISTOGENESIS

IHC methods have brought about a revolution in approach to diagnosis of tumours of uncertain origin, primary as well as metastatic from unknown primary tumour. A panel of antibodies is chosen to resolve such diagnostic problem cases. Immunohistochemical stains for intermediate filaments are expressed by tumour cells (keratin, desmin, vimentin, neurofilaments, and glial fibrillary acidic proteins).

PREDICTION OF RESPONSE TO THERAPY

IHC is widely used to predict therapeutic response.

The specific receptors for these growth regulating hormones are located on respective tumour cells. Tumours expressing high level of receptor positivity would respond favourably to removal of the endogenous source of such hormones or hormonal therapy is administered to lower their levels

INFECTIONS

Immunohistochemical methods are also being applied to confirm infectious agent in tissues by use of specific antibodies against microbial DNA or RNA, e.g. in Cytomegalo virus, Hepatitis B virus, Hepatitis C virus, etc

IN GENETICS

IHC can also be used to determine the function of specific gene products in fundamental biological processes such as development and apoptosis. Using a custom made monoclonal antibody against p53 homologue of the pro-apoptotic pathways of p53 was identified.

NEURODEGENERATIVE DISORDERS

Degenerative disorders of the nervous system include a wide range of diseases characterized by the dysfunction and death of specific, selectively vulnerable populations of nerve cells. It has played an increasingly important role in the sub classification of neurodegenerative disorders and the development of consensus criteria for their diagnosis.

BRAIN TRAUMA

Immunohistochemical staining for beta amyloid precursor protein has been validated as a method to detect axonal injury within as little as 2–3 h of head injury. Immunohistochemical detection of axonal injury can be useful in establishing timing of a traumatic insult in medico-legal settings.

IHC IN MUSCLE DISEASES

Specific diagnosis of muscular dystrophy is important because of the genetic counselling implications of inherited disease and accurate prognostication. Skeletal muscle biopsy can play a main role in differentiating vascular dystrophy from non-dystrophic disorders and IHC can assist in establishing a specific diagnosis of the dystrophies for which specific protein abnormalities are known.

RESEARCH APPLICATION

Studies to localize and quantify the abnormal proteins that constitute reasons of neurodegenerative diseases are of central importance. IHC using antibodies to beta amyloid, alpha synuclein, ubiquitin, huntingtin, polyglutamine, and others has become a routine tool for a sensitive detection and quantification of these abnormal proteins in both human tissues and in experimental animals that are used to model some of the features of these diseases. IHC is an important tool in diagnostic and research laboratories.

6) RECENT ADVANCES AND FUTURE DIRECTIONS:

Rapid immunohistochemical investigation in addition to staining with haematoxylin and eosin would be useful during intraoperative frozen section diagnosis in some cases by using EnVision Antibody Complex.A broad spectrum of antigens could be detected in frozen sections within less than 13min, using a modified protocol for the EnVision system.[16]

Several recent developments emphasize the increasingly important role immunohistochemistry will play in the coming years. These include genogenic immunohistochemistry for diagnosis, search for proteins for targeted therapy, methods to develop better monoclonal antibodies with recombinant technology, "technician-free" automation of the IHC procedures, and "pathologist-free" microscopic image analysis technology for interpretation of high-throughput results.[18]

"Genogenic immunohistochemistry" heralds a new era in immunohistochemistry, and identification of the underlying molecular changes by immunohistochemistry is being used both for diagnosis and therapy.[17]

Automation in IHC has been advocated for carrying out the procedures for consistency in performance. Methods using automated computerized image capture and analysis systems as opposed to the traditional subjective observations of IHC stains are being introduced. The emergence of tissue microarrays (TMA) as a high-throughput technique for examining hundreds of marker molecules in histological microarray sections comprising between 100 and 1,000 core tissues on a single glass slide enables economical evaluation in terms of sample utilization and reagent costs.[18]

In future, TMA will be an increasingly sought-after tool for evaluating the expression of proteins by IHC and thus validating the findings of DNA microarrays.[18]

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