

# Journey of Immunohistochemistry in General and Oral Pathology: A Review on its Applications and Updates

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**Abstract:** Immunohistochemistry is a technique with an interesting journey. It is one of the well-established and reliable technique in modern pathology and it is extremely useful in diagnosis of sinister pathologies with perplexing histopathology. Not only does it enhance the diagnostic abilities of a pathologist, but it also has a huge prognostic potential, a great aid in establishing stage of malignancies, determines phenotypic expressions of lymphoid neoplasms and monitors treatment progress and response to therapy. By employing and integrating the basics of varied branches like immunology, histology, microscopy and hematology, it has emerged as a magnificent tool over last few decades, saving pathologists and patients from the impact of serious diseases inflicting the human body. Furthermore, it has contributed immensely to all aspects of diseases related to oral cavity as well. This review has been thus taken to highlight the wide applications of this technique in General and Oral Pathology with an update.

**Keywords:** Applications; Immunohistochemistry; Oral; Pathology; Update

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## 1. Introduction

Immunohistochemistry (IHC) is one of the well-established techniques in fields of disease investigations, diagnosis of various neoplasms, study of tumor progression, prognosis of different pathologies, establishing pathogenetic mechanisms, and even monitoring cancer therapy. It has widest applications in establishing the nature and origin of neoplasms affecting body. It also aids the diagnosis of oral neoplasms and thus helps in instituting appropriate treatment strategy which can save patients' lives. It makes use of the basic mechanism of the reaction between antigens and antibodies and their complexes, followed by visualizing it under an optical microscope with the use of color tags. Thus, antigenic markers pertaining to specific tumor antigens can be determined using IHC. The technique has been applied extensively in diagnosis of various oral tumors of different lineages by identifying their particular markers. Extensive research in recent times has shown its role in identifying markers of cell proliferation and growth of oral tumors. It also helps in looking for different biological pathogens in infectious oral diseases. Moreover, oral neoplasms of uncertain origins posing great diagnostic dilemmas to oral pathologists can be revealed through their

histogenetic origins with the aid of IHC. It also helps in locating roots of primary as well as metastatic tumors of oral soft tissues and jaws. IHC has revolutionized oral cancer research through its far-flung manifestations. This review will discuss the technique briefly with its superfluous role in oral pathology, its wide applications in oral diseases and tumors along with recent update.

## **2. Historical evolution**

Though it is known since 1931, Coons et al. in 1941 were the pioneers who carried out this technique for the first time in identification of antigens of pneumococcus bacteria using an antibody labelled with fluorescent dye<sup>[1,2]</sup>. These fluorescent antibodies were then developed in laboratories and were then applied on a larger scale in the 1960s. These antibodies had their own limitations like poor morphological details, interference in visualization by tissue fluorescence and inability to archive these fluorescent labelled slides. Drawbacks related to these fluorescent antibodies pushed the researchers to look for better alternatives. Various metal containing proteins like ferritin and certain enzymes were experimented as labels for the antibodies in place of fluorescent dyes. Success was achieved by Nakano and Pierce in 1960 for using horseradish peroxidase as an easily available and cheap substitute of fluorescent dye. 3,3'-diaminobenzidine (DAB) when used as a substrate along with it could readily identify the enzyme<sup>[3]</sup>.

A histochemical method which employed peroxidase antiperoxidase (PAP) and DAB to detect the antibodies in the complex was developed by Sternberger et al. in 1970. They used an additional antibody which is now called as secondary antibody against the first antibody which appeared as brown complex in sections under light microscope when further stained with PAP and DAB, the technique later came to known as PAP technique<sup>[4]</sup>.

In pursuit of finding new labels, researchers gradually developed the technique and expanded its dimensions by furnishing efficient enzyme labels like peroxides and alkaline phosphatase, colloidal gold etc. IHC reactions developed with the use of these markers were sufficiently strong to be visible in light and electron microscopes<sup>[5-7]</sup>. Recent development of these immunological markers has led to the emergence of immunohistochemistry in the field of head and neck pathology diagnosis as an important tool used in routine histopathology. Though normal traditional staining method using Hematoxylin and Eosin is largely applied even now, IHC has emerged as the most important resolve for cases where origin of tumor cells become enigmatic.

## **3. Applications of immunohistochemistry**

As a well-established discipline, IHC has evolved by applying concepts in immunology, hematology, and microbiology to histologic techniques. During the past 30 years, IHC has progressed from a novel idea in research to a widely accepted adjunct to the routine histologic work-up of tissues and cytologic preparations. IHC is useful in assessing prognosis, monitoring organ transplants, determining the stage of tumors, establishing the phenotype of lymphomas, and providing clues to a patient's probable response to therapy. The technique also has widespread application in pharmaceutical and cancer research.

Furthermore, it plays important roles in different areas of dental diagnoses pertaining to head and neck region. This technique mainly stands out for diagnosis of difficult oral neoplasms, along with disease progress assessment and monitoring treatment protocols. It has major role in surgical pathology cases where it distinguishes tumors of epithelial origin from those of connective tissue origin. Carcinomas can be differentiated from sarcomas, lymphoid tumors and melanomas<sup>[8]</sup>. Various research laboratories and clinical set ups are developing the technique due to its crucial role in diagnosis<sup>[9]</sup>. In this review, we will discuss briefly about the role of immunohistochemistry and the markers employed in different pathologies of the body in general and those pertaining specifically to the oral region.

#### 4. Prognosis of neoplasms

The technique helps in recognizing specific antigen types of tumors, genes associated which can be oncogenic or suppressor genes, and certain markers that increase during proliferation phase of cells. By knowing these markers, a better tumor prognosis can be established as compared to traditional evaluation prognosis based on clinical picture and grade of histopathology. This can further enhance the horizon by helping in development of medicines and research of biological factors associated with tumors. This can be done by targeting the factors which helps us in disclosing whether disease progression or remission occurs following use of certain drugs based on identification of tumor markers <sup>[10]</sup>.

#### 5. Monitoring therapy for neoplasms

Certain tumors when treated with hormones like that of malignancy of breast and prostate can be monitored for treatment effectiveness. In these neoplasms, IHC can help in localizing the increased receptor expression of specific receptors of these hormones against the related tumor cells. Thus, neoplasms where there is manifold increase of these receptors can be assessed through immunohistochemistry, and this is helpful in predicting the response of such neoplasms towards a treatment <sup>[10]</sup>. This can further help in identification of potential target sites for tumor therapy <sup>[8]</sup>.

#### 6. Role in genetics

IHC can also help in identification of gene mutations <sup>[8]</sup>. The probable mechanism by which certain products produced by specific genes act can be delineated at a cellular level through processes like apoptosis and normal development. For example, monoclonal antibodies against p53 can be produced and utilized by IHC in studying p53 expression in pathway of process of apoptosis <sup>[10]</sup>.

#### 7. Immunohistochemical detection of infections

It is a technique which has an elaborative role in diagnosis various types of infectious agents like bacteria, viruses, fungi and parasites. It is done through detection of pathogenic antigens with the help of monoclonal and polyclonal antibodies against them <sup>[11]</sup>. Different bacteria which can be detected through immunohistochemical procedures are *Bartonella quintana*, *Yersinia pestis*, *Treponema pallidum*, *Chlamydia trachomatis*, *T. whipplei* and *C. burnetii*, *S. aureus*, Group A *Streptococci* etc. Cross reaction between bacterium *M. tuberculosis* antibody and normal eosinophil granules can occur and IHC technique has been proven to be of great aid in identification of skin mycobacteriosis <sup>[12-14]</sup>. Other infections caused by bacterial species of *Clostridium*, *Chlamydia pneumoniae*, *Neisseria meningitidis*, *Borrelia burgdorferi*, *Rickettsia rickettsii*, *Burkholderia pseudomallei*, *Orientia tsutsugamushi*, *Helicobacter pylori*, *Brachyspira* species can also be detected with this technique <sup>[11]</sup>. Various viruses that can be identified are human herpesvirus, human immunodeficiency virus (HIV), enterovirus etc. It has also helped in understanding the pathogenesis of viral diseases like Middle East respiratory syndrome (MERS), influenza A (H1N1) and B virus infection. It is also largely useful in diagnosis of yellow fever, hepatitis C virus infection, infections associated with Zika virus, West Nile virus, adenovirus, Hantavirus, skin infections caused by viruses like parvoviruses, paramyxoviruses and poxviruses <sup>[11]</sup>. Various fungal diseases that can be localized through immunohistochemistry include aspergillosis <sup>[15]</sup>, sporotrichosis <sup>[16]</sup>, pythiosis <sup>[17]</sup> and fungal sinusitis <sup>[18]</sup>. It can also serve as an accurate diagnostic method for identification of *Candida albicans* and *Cryptococcus neoformans* <sup>[11]</sup>. Amongst protozoal infections, diagnosis of toxoplasmosis, malaria and echinococcosis and detection of *Trypanosoma cruzi* are significantly improved by use of Immunohistochemistry <sup>[11]</sup>.

## **8. Role in neurodegenerative disorders and cerebral trauma**

IPC plays a role in understanding and thus classifying disorders related to degeneration of nervous system of body which may constitute a wide array of disorders related to death and changes in functioning of cells of nervous tissue. Extending the scope, it further helps in the formation of a consensus criterion to establish an accurate diagnosis. In cases of human head trauma, axonal injury can be identified through staining of beta amyloid precursor proteins within a short span of 2–3 hours of trauma <sup>[19]</sup> which can be hugely useful in estimating the time of trauma for medicolegal purpose <sup>[10]</sup>.

## **9. Diseases of muscles**

In cases of dystrophies of muscle, various proteins of muscles present in its matrix, cytoplasm, sarcolemma and nuclear sites are involved which can be site localized through immunohistochemical process that helps in establishing a specific diagnosis <sup>[20]</sup>. This further plays an important implication in genetic counselling and prognostication for such dystrophies.

## **10. Neoplasms with perplexing origins and metastasis**

There are various neoplasms which can perplex pathologists. IHC has been a tremendous help for pathologist in such difficult situations. Primary tumors as well as metastatic tumors without known primary sites can be approached well through IHC as the cell of origin can be delineated through identification of specific antigens by IHC evaluation. After obtaining the history, clinical details, histopathology report and other investigative procedures records, certain antibodies can be selected as markers which are expressed by different neoplastic cells <sup>[10]</sup>.

## **11. Immunohistochemistry revolution in diagnosis of head and neck tumors**

### **11.1. Oral squamous cell carcinoma**

The most concerning malignancy of this region is oral squamous cell carcinoma. Keratins are the main epithelial proteins which are released by these epithelial tumor cells. Markers against these keratins can be used for diagnosis of these tumors. As these tumor cells reach connective tissues and can metastasize, changes produced by these cells in stroma can also be studied through IHC to understand the pathogenesis and biological behavior of this malignancy. As tumor cell proliferation occurs, certain antigens of cell proliferation can also be assessed to understand the biological potential of these tumors <sup>[21]</sup>. The behavior of potentially malignant disorders can also be predicted based on immunohistochemical expression of prognostic markers, cyclin D1, p27 and p63. Ki-67 is used as proliferation marker. Another such marker, proliferating cell nuclear antigen (PCNA) can be used to monitor treatment response in oral squamous cell carcinoma cases as its score decreases post chemotherapy <sup>[22]</sup>. Research at large scale can help in establishing a grading system for oral epithelial dysplasia which in turn can aid in early detection of possible malignant transformation <sup>[23]</sup>.

### **11.2. Melanomas of oral cavity**

This tumor may show enormous variation in its histopathology and has a characteristic antigenic expression that helps in its differentiation with immunohistochemical positive expression for S-100, neuron specific enclave and vimentin; negative for keratin protein <sup>[21]</sup>. Homatropine methylbromide-45 marker positivity especially in neurotropic melanoma helps in its differentiation from oral soft tissue sarcomas that it simulates <sup>[22]</sup>. Melanin producing melanoma of soft parts can be distinguished from synovial sarcomas in being positive for melanin and negative for mucin immunohistochemically <sup>[24]</sup>.

### **11.3. Salivary gland neoplasms**

The application of IHC on salivary gland neoplasms is limited as they have diverse histopathology and features. The technique helps in typing of salivary gland neoplasms in a few cases which helps in diagnosis and prognosis. Pleomorphic adenoma, the most common benign tumor of salivary glands can be differentiated from other benign conditions and polymorphous low-grade carcinoma with positive Glial fibrillar acidic protein marker <sup>[25]</sup>. S-100 protein, actins and epithelial membrane antigen are used as immunohistochemical markers for diagnosis of adenoid cystic carcinoma aids as they are expressed in limited pattern in this tumor.

### **11.4. Oral connective tissue neoplasms**

Benign connective tissue tumors that usually require immunohistochemical help in differentiation from similar lesion with different origins are tumors with granular cells, neurilemmoma and neuroectodermal tumor of infancy. Granular cell tumor shows positive expression for S-100 and vimentin like neurilemmoma but can be differentiated from muscle tumors as it remains negative for markers for muscle proteins, actins and desmin <sup>[21]</sup>. In contrast, another tumor with granular cells, granular cell tumor of gingiva of newborns shows positive expression for vimentin and negative for S-100 protein relating to its fibroblastic origin <sup>[26]</sup>. Sarcomas arising in oral cavity can be confused with carcinomas like sarcomatoid spindle cell carcinoma and malignant melanoma. Thus, markers like vimentin are used for which majority of sarcomas show positive expression and keratin remain negative <sup>[20]</sup>. The most common soft tissue sarcoma of maxillofacial region, embryonal rhabdomyosarcoma in children requires diagnostic considerations from other tumors like Ewing's sarcoma, synovial sarcoma, melanoma, melanoectodermal tumor of infancy and lymphomas. Desmin is a myogenic selective marker that helps in relieving this concern by being present in almost all cases of embryonal rhabdomyosarcoma and absent in small round cell tumors <sup>[27]</sup>. Certain regulatory proteins like MyoD1, myogenin, Myf-5, and Myf-6 are highly useful in detecting poorly differentiated cases of rhabdomyosarcoma <sup>[28]</sup>. For osteosarcomas, recognizing osteoblast and tumor osteoid are the main frameworks for diagnosis for which two markers, osteocalcin and osteonectin have been hugely beneficial. Usage of monoclonal antibodies for osteocalcin shows high positivity for specific bone forming cells and tumors <sup>[21]</sup>.

### **11.5. Malignancies of blood vessels**

Vascular tumors with poorly differentiated variants and tumors with different origin but showing similar histopathology to angiosarcomas pose great difficulties in their diagnosis. CD31, platelet endothelium cell adhesion molecule-1 is most useful in such cases which act as endothelial cell immunohistochemical marker which is found to be positive in about 90% cases of angiosarcomas <sup>[28]</sup>. A recently identified nuclear transcription factor, FL11, has been found to be positive in almost all cases of vascular tumors.

### **11.6. Oral lymphomas**

IHC plays an imperative role in diagnosis oral lymphomas. Reactive lymphoid proliferations are differentiated from small cell lesions by delineating their clonal nature. Antibodies against LCA, CD45, keratin and S-100 are used to differentiate large cell lymphomas from undifferentiated malignancies like carcinomas and melanomas. More specific markers for lymphoid tissue, T lymphocytes (UCHL-1, CD3, L60, and MT1) and B lymphocytes (L26, LN1, LN2, LMB1, and MB2) are further used <sup>[29]</sup>. Important marker for B-cell, CD20, and other pan B-cell antigens, CD19 and CD22 can be identified in B-cell lymphomas. CD 10 positive expression is seen in Burkitt's lymphomas <sup>[21]</sup>. Reed Sternberg cell markers, CD15, CD30, CD45 and BLA36 can be used for Hodgkin's lymphomas <sup>[29]</sup>.

## 12. Conclusion

Immunohistochemistry technique has developed to a great extent in recent years. It is applicable in all aspects of disease process, from prediction of premalignancy to diagnosis of uncertain malignancies to identification of targets for successful tumor therapy. Further research in technology and exploration of its use to expand the horizon of its applications can be a boon to the fields of General Pathology and Oral Pathology.

## Disclosure statement

The authors declare no conflict of interest.

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