Review Article

Expression sites of immunohistochemistry markers in oral diseases – A scoping review

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Abstract

Introduction: Immunohistochemistry (IHC) has not always been an easy field for the research beginners like postgraduates, research fellows and scientists. Meaningful interpretation of IHC positivity needs expertise. This could be made easier for beginners by developing a conceptual framework of markers. The literature review revealed a lack of qualitative evidence on the hitherto IHC studies on oral diseases about the overall expression of IHC markers and its comparison with pathology and normal tissues.

Aim: This scoping review aimed to examine the literature and classify the various immunohistochemistry markers of oral diseases based on the tissue, cell and site of expression.

Materials and Methods: The review was in accordance with Preferred Reporting Items for scoping reviews (PRISMA -ScR). Electronic databases such as PubMed and Cochrane were searched for relevant articles till 2021.

Results: We included 43 articles. We found five different possibilities of the site of expression of a marker in a cell. They are the nucleus, cytoplasm, cell membrane, extracellular matrix or any of the above combinations. Based on the tissue of expression, we also mapped the markers expressed in oral diseases to their tissue of origin as ectoderm, endoderm, mesoderm and markers with multiple tissues of expression. Based on our results, we derived two classifications that give an overview of the expression of IHC markers in oral diseases.

Conclusion: This scoping review derived new insight into the classification of IHC markers based on cell lineage, tissue and site of expression. This would enable a beginner to better understand a marker with its application and the interpretation of the staining in research. This could also serve as a beginner's guide for any researcher to thrive and explore the IHC world.

Keywords: Biomarkers, classification, immunohistochemistry, markers, oral disease

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INTRODUCTION

Most practitioners, including beginners in pathology and research, are familiar with Immunohistochemistry (IHC) and its applications. Immunohistochemical markers are the various antibodies that are employed in IHC and the constant upgrading in the field of IHC has led to the surge of IHC

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markers in the past decades.[1] Although there are various diagnostic algorithms and classifications of IHC markers, the practical knowledge of any graduate or a beginner in terms of the interpretation of IHC positivity is of great concern. The most common grey area of any beginner while IHC slide interpretation would be, what are all the cells that can be positive for the IHC markers applied? And where to look for positivity (Nucleus/cytoplasm/cell membrane)? Our literature search revealed numerous immunohistochemical studies that discuss and classify the IHC markers[2-4] but none of them reviewed and classified the markers based on their site and tissue of expression. Hereby, we planned to study extensively the literature to understand the markers based on tissue of origin and site of expression and to classify them.

AIM AND OBJECTIVES

We aimed to systematically review the literature and explore the IHC markers studied in oral diseases. Our objectives were to.

1. Compile the markers with their tissue, cell and site of expression in the normal and pathology.
2. Classify and categorize the markers based on the tissue, cell and its site of expression in a cell.

METHODOLOGY

This scoping review was conducted in accordance with PRISMA for scoping reviews [Figure 1]. Electronic databases such as PubMed and Cochrane were searched for studies with IHC markers expressed in oral diseases.

Scoping review question
The topic of interest was the expression sites of IHC markers of oral diseases and the research question was:

What are the various expression sites (cell and tissue) of IHC markers of oral diseases? This research question encompasses the expression of IHC markers at various
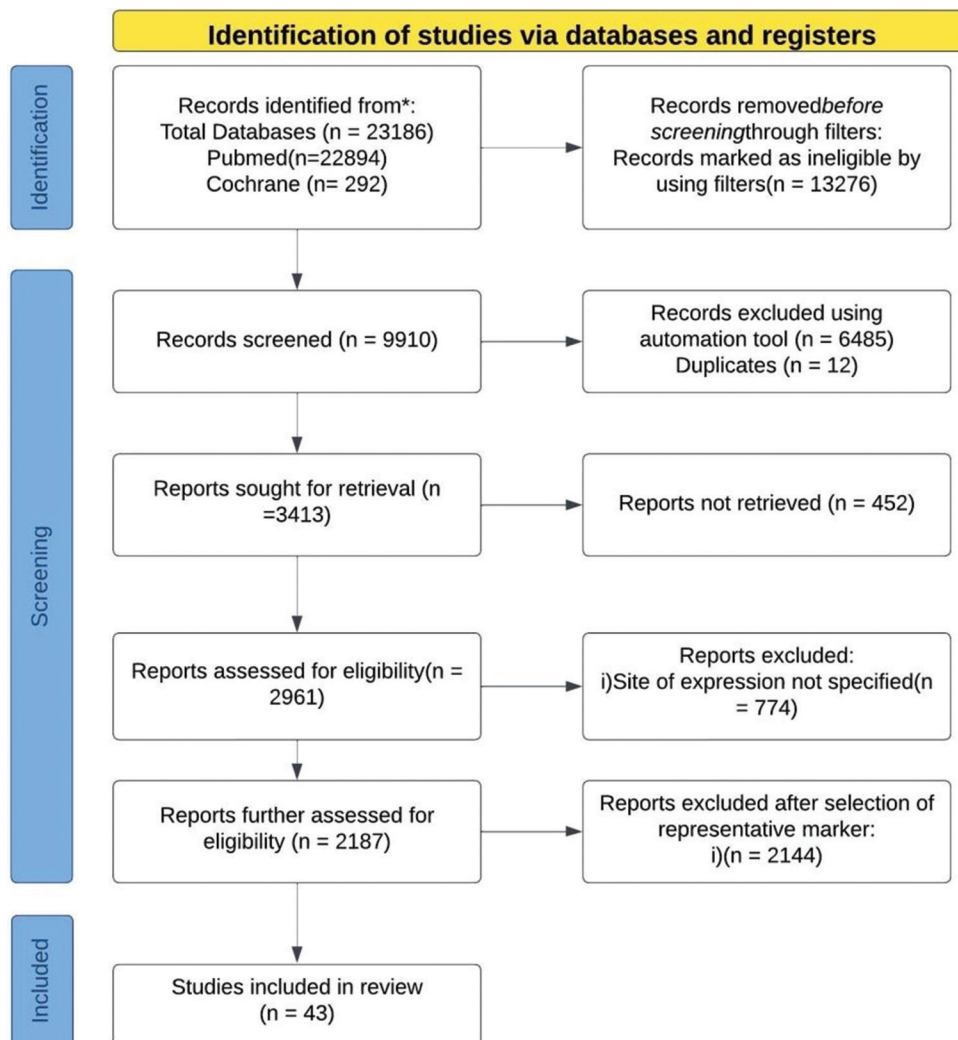

Figure 1: PRISMA-ScR, search flow chart for scoping review

tissues of normal and oral pathologies with respect to their cell site of expression.

Search strategy

A team of six reviewers was involved in the review process. The various stages that were involved were the title and abstract screening, full-text retrieval, data extraction and data analysis. We used the Sample, Phenomena of Interest, Design, Evaluation, Research type (SPIDER) framework to identify the keywords in the review question. Once the keywords are ascertained, a table listing all the synonyms and MeSH terms were developed to guide the search. A Boolean search string was developed combining the plain text and MeSH terms as follows:(((Oral OR Dental) AND (Marker OR Immunomarker OR Biomarker OR Immunohistochemical Marker OR Tumour Marker)) AND (Expression OR Immunohistochemical demonstration OR Immunohistochemical localization OR Immunohistochemical characterization))).

Title and abstracts were screened for potentially eligible studies. An automation tool was used to remove the duplicates. Two independent reviewers read the full article for relevance to the research question. When found relevant, the interesting data were extracted.

Data extraction

Two authors extracted data from the selected articles. The collected data were entered in a data collection form in a standardized manner using Excel spreadsheets. Data were gathered on the first author, publication year, country and publication language and then the data required to answer our research question like the pathology studied, control (normal tissue) taken, markers applied, tissue, cell and site of expression markers in pathology, as well as in normal, were collected. The studies were categorized based on tissue of expression mapping to their embryologic origin. Later, each of the studies with IHC markers was analysed for the site of expression in the cell. The data analysis was undertaken by the fifth and sixth reviewers. Any discrepancies that were found were resolved by the sixth reviewer for a decision.

Inclusion criteria

The articles from PubMed and Cochrane databases that were in the year between 1992 and 2021, English language, original research, observational study, clinical study and randomized control trials in humans were considered for inclusion. Studies with IHC markers studied on human oral diseases that mention the tissue, cell and site of expression of markers.

Exclusion criteria

Reviews, reports, letters to the editor and studies with methods other than IHC such as ELISA, western blot, FISH, cell culture and study duplicates were excluded. Also, the unavailable full-text articles, articles that do not discuss the site of expression of markers, and articles with repeated markers were excluded.

RESULTS

The primary search using the Boolean search string generated 23,186 articles, which were published between 1992 and 2021. When the filters such as original research, observational study, clinical study and randomized control trials in humans were applied, 9910 articles were generated and they were considered for screening. Using an automation tool, all the 9910 articles were screened for the inclusion and exclusion criteria. In this stage, we excluded (n = 6485) and duplicate studies (n = 12), and only 3413 articles were included for the full-text retrieval. Out of them, only 2961 articles were available as full text. All the 2961 full-text articles were read thoroughly for the markers used, and their tissue, cell and site of expression in the cell were noted. Among the 2961 articles, 774 articles were excluded as they did not mention/discuss the site of expression. Thus, we obtained 2187 articles and further observed that multiple studies studied the same marker in various pathologies. Therefore, we choose 43 studies with representative markers under each category and excluded the rest 2144 studies [Figure 1]. The time needed for the completion of the scoping review was approximately 8 months.

All 43 studies were analysed for the expression characteristics of markers such as the tissue, cell and the site of expression in a cell in the pathology as well as in the control tissues [Table 1]. A total of 46 IHC markers that represent each category were analysed. Out of them, 3 were categorized under Primitive Germ cell markers, 14 markers were expressed in ectodermal/endodermal derived oral tissues such as epithelium (3), nerve (2), bone and cartilage (1), melanocyte (3), mesenchyme (2), Muscle (2) and Dentin (1), and another 10 were expressed in mesodermal-derived structures such as Tongue Muscle (2) myeloid (3), lymphoid (3) and endothelial (2). Later, there were 19 markers expressed by multiple tissues structures of various tissue of origin namely Adult stem cell markers (1), nuclear membrane markers (1), nucleolar marker (1), chromatin marker (1), genetic markers (1), Cell cycle markers (1), apoptotic markers (1), enzymes (2), transmembrane markers (1), cell surface/adhesion markers (2), cytokine marker (1) calcium-binding

Table 1: Expression of markers in pathology and normal tissue with the site of expression in a cell

Category	Author	Marker	Pathology			Normal		
			Pathology	Cells	Site of expression	Tissue	Cells	Site of expression
Primitive Germ cell Markers								
Germ Cell markers	Maria Fernanda Setubal Destro Rodrigues et al.[5]	Oct 4	MEC	Tumour cells – cells coating cystic spaces	C&N	NSG	Ductal cells and acinar cells	C
		Nanog	MEC	Tumour cells – cells coating cystic spaces	C&N	NSG	Ductal cells and acinar cells	N
	Fu-fang Wang et al.[6]	Carcinoembryonic antigen (CEACAM1)	SCC	Tumour cells – epithelial cells	CM&C	NOM	Stratified squamous cells	CM
Markers expressed in Oral tissues of Ectoderm/Endoderm origin								
Epithelial markers	Partheeban Balasundharam et al.[7]	E- cadherin	OSCC	Tumour cells	CM	Oral buccal mucosa	Basal and suprabasal cells	CM
	Jean Carlos Barbosa Ferreira et al.[8]	MUC1	PA	Ductal epithelial cells	C	Minor Salivary Gland	Ductal epithelial and Acinar cells	C
	HW Gao et al.[9]	CK20	BSL, VC & SCC	(Merkel cells) basal layer	C	Adjacent NOM	Merkel cells	C
Nerve markers	Douglas R. Gnepp et al.[10]	GFAP	PLGA	Epithelial cells	C	Normal Control was not taken		
		GFAP	Benign Mixed Tumour	Epithelial and stromal component – Duct cells, Periductal cells, plump to spindled mesenchymal-like cells	C	Normal Control was not taken		
Bone and Cartilage markers	P Nankivill et al.[11]	CD82	OED	Dysplastic epithelial cell - Spindle shaped cells	CM	Normal control was taken		
	El Achkar VNR et al.[12]	Osterix	POF	around mineralized tissue, -Entrapped osteocytes and -Foci of mineralized Tissue	N	Normal control was taken		
Melanocyte markers	Jie Zeng et al.[13]	MIF	OSCC	Tumour cells	C/CM	Normal oral mucosa		
	Bruno- Augusto- Benevenuto de – Andrad et al.[14]	HMB-45	Primary oral melanoma	Melanoma cells	C	No normal control was taken		
		Melan A	Primary oral melanoma	Melanoma cells	C	Normal control was not taken		
Mesenchymal markers	Vered M et al.[15]	Fibroblast-specific protein (FSP)-1	OSCC of Tongue	Tumour cells	C and N	No control was taken		
	Partheeban Balasundaram et al.[7]	Vimentin	OSCC	Tumour cells	C	Oral Buccal mucosa	Mesenchymal connective tissue cell	C
	Bruno D Sedassari et al.[16]		PLGA	Tumour cells	C	No normal control was taken		
Muscle marker	Matsuzaki Y et al.[17]	Actinin 4	OSCC	Tumour cells	CM	Normal control was not taken		
	Smitha A et al.[18]	α SMA	OSCC	Myofibroblasts in Tumour Stroma	C	NOM	Smooth muscles of blood Vessels	-
Dentin marker	Kalu U.E. Ogbureke et al.[19]	DSPP	OPL with Dysplasia	Epithelial dysplastic cells	C	No normal control was taken		
Markers expressed in Oral tissues of Mesoderm origin								
Tongue Muscle	FernandaSalgueiredo – Giudice et al.[20]	SMA	Inflammatory myofibroblastic tumour of Tongue	Myofibroblasts	C	Blood vessels	-	-
		MSA			C	Normal control was not taken		-

Contd...

Table 1: Contd...

Contd...

Category	Author	Marker	Pathology			Normal		
			Pathology	Cells	Site of expression	Tissue	Cells	Site of expression
Markers expressed in Oral tissues of Mesoderm origin								
Myeloid marker	Celeste Sánchez-Romero et al.[21]	GLUT-1	Ab	Ameloblastic cells, Stellate reticulum cells	CM/C	Tooth Germ	Ameloblastic (Epithelial cells)	CM/C
	Hala H. Hazzaa et al.[22]	CD163	HGF	Macrophages in connective tissue	C	Gingival tissue	Sub-epithelial connective tissue cells	C
	Soudabeh Sargolzaei et al.[23]	CD68	PGCG	Multinucleated giant cells, mononuclear cells and endothelial cells	C	Internal control – germinal centre of Lymph node and blood vessels in the periphery of lesions		
Lymphoid markers	G.T. Peterle et al.[24]	FASL	OSCC	Lymphoid cells –inflammatory infiltrate	CM	No normal control was taken		
Endothelial markers	L. A. Aqrawi et al.[25]	CD20 , CD27	SS	Ductal or acinar cells	CM	NSG	Ductal or acinar cells	CM
	Anji Anura et al.[26]	VEGF	OSMF	Epithelial cells	C & ECM	NOM	Epithelial cells	Stromal and Cytoplasmic expression
	Gino Marioni et al.[27]	Endoglin (CD105)	Oropharyngeal SCC	Tumour endothelial cells	C	Adjacent normal mucosa	Endothelial cells	-
Markers of Multiple Tissue of Expression								
Adult stem cell marker	Maria Fernanda Setubal Destro Rodrigues et al.[5]	Bmi1	MEC	Tumour cells	N	NSG	Ductal cells and acinar cells	N
Nuclear Membrane Protein	Salvh M. Rachidi et al.[28]	KPNA2	HNSCC	Tumour Cell	C & N	NOM		
Small Nucleolar Ribonucleo protein	Li HG et al.[29]	IMP3	OSCC	Tumour Cell	C	NOM	Epithelial cells	-
Chromatin Markers	Paulo E. A. Desouza et al.[30]	MDM 2	CGCG & GCT	Tumour cell	N	Normal control was not taken		
Genetic marker	Pablo Rosado et al.[31]	P 53	OSCC	Tumour cells	N	Normal control was not taken		
Cell Cycle markers	Hiroyuki Kumamoto et al.[32]	Cyclin D1	Ab	Tumour epithelial cells	N	Tooth Germ, Normal gingiva	Epithelial cells	N
Apoptotic marker	Gatumu MK et al.[33]	BCL 10	Primary SS	Infiltrating Lymphocytes	N	Tonsil, Lymph nodes	Germinal Centre cells	C
Enzyme Marker	Punnya V. Angadi et al.[34]	PTEN	OSMF	Epithelial cells	N	NOM, Blood vessels	Epithelial cells, Endothelial cells	N
	Ying-Wen Su et al.[35]	Phosphorylated AMP-Activated Protein Kinase (pAMPK)	HNSCC	Tumour cells	C	NOM		N
Trans-membrane protein	Wagner Gomes da Silva et al.[36]	Syndecan 1	Central CCOT	Stellate reticulum and Basal cells / Ghost cells	CM / C	Normal oral epithelium		CM

Table 1: Contd...

Category	Author	Marker	Pathology			Normal		
			Pathology	Cells	Site of expression	Tissue	Cells	Site of expression
Markers of multiple Tissue of Expression								
Cell surface/ adhesion markers	Maria Fernanda Setubal Destro Rodrigues et al.[5]	CD44 (HCAM)	MEC	Clear cell, Intermediate and epidermoid cells	CM	NSG	Ductal cells and Acinar cells	CM
	Upadhaya Pet al.[37]	JAM A	OED	Tumour cells	C, CM	Normal epithelium	Epithelial cells	CM
Cytokine marker	Samapika Routray et al.[38]	Osteopontin (OPN)	OSCC OSCC	Tumour cell Tumour cells	C C	NOM	-	-
Calcium binding protein	Sapkota D et al.[39]	S100A16	OSCC	Tumour cells	C	NOM	Epithelial cells	CM
Cytoplasmic Inclusion marker	Yuichiro Honjo et al.[40]	Galectin 3 (Carbohydrate binding Molecule)	OSCC of Tongue	Tumour epithelial cells	N, C	NOM	Epithelial cells	N & C
Cytoplasmic Organelle Marker	Kaushik Kumar Dey et al.[41]	RAB2A (Endoplasmic reticulum)	OSCC	Tumour cell	C, N	Adjacent normal tissue		
ECM markers	P. Papagerakisl et al.[42]	Type IV collagen	Mixed odontogenic tumours	Tumour epithelial cells	C, BM	Normal control was not taken		
	S. Mori et al.[43]	HS-GAG	SCC	Carcinoma cell	C	Squamous epithelium, Blood vessels, Smooth muscle of vessels	Keratinocyte	BM and Subepithelium
	Ivika Luksic et al.[44]	Fibronectin	Early stage OSCC	Stromal Cell	C	Normal control was not taken		

Ab - Ameloblastoma, BSL - Benign Squamous Lesion, CCOT-Calcifying Cystic Odontogenic Tumour, GCT - Ghost Cell tumour, HGF - Hereditary Gingival fibromatosis, MEC - Mucoepidermoid carcinoma, NOM - Normal oral mucosa, NSG -Normal salivary gland, OED - Oral Epithelial dysplasia, OPL - Oral premalignant lesions, OSCC - Oral squamous cell carcinoma, OSMF - Oral submucous fibrosis, PA - Pleomorphic adenoma, PGCG - peripheral Giant cell Granuloma, PLGA - Polymorphous low-grade adenocarcinoma, POF - Peripheral ossifying fibroma, SCC - Squamous Cell Carcinoma, SS - Sjogren syndrome, C- Cytoplasm, CM - Cell Membrane, N - Nucleus, BM - Basement membrane, HNSCC - Head and Neck squamous cell carcinoma, VC - Verrucous carcinoma

proteins (1), cytoplasmic inclusions (1), cytoplasmic organelle (1) and extracellular markers. (3) [Table 1].

DISCUSSION

The Success of an immunohistochemical diagnosis depends on the thorough knowledge of the various markers, their cell lineage and site of expression to apply in the field of research and clinical diagnosis. Although there are numerous classifications of IHC markers based on the cell types, biochemical class, diagnostic relevance and so on,[2-4] none of them discuss and classify the markers based on the tissue, cell and site of expression which would be useful for a beginner. Based on the tissue expression of the IHC marker and its cell lineage, we have classified the markers under four broad categories. They are the markers that are expressed in the tissues that are of:

1. Primitive germ cell origin
2. Ectodermal origin: Oral epithelium and cranial neural crest cells derivative CNCs give rise to two main structures called Ectomesenchymal and non-ectomesenchymal structures.[45]
   Tissues such as oro-facial bone, cartilage, muscle, ligament, fascia, tendon, fat,[46] dentin, pulp[47] and mesenchyme[46,48] are ectomesenchymal derivatives of CNC. Whereas, the orofacial neurons and melanocytes are the non-ectomesenchymal derivative of CNCs.[49] The oral epithelium is majorly ectodermal in origin. This includes the epithelium of lips, floor of the mouth, gingiva and cheeks.[50] Also, recent studies have proposed that all the major salivary glands and epithelium of the anterior tongue and fungiform papillae and hard palate are of ectodermal origin.[45,51] The minor mucosal salivary glands are of both ectodermal and endodermal origins.[45]
3. Endodermal origin: Epithelium of the posterior third of the tongue, floor of the mouth, palatoglossal folds and the soft palate,[51] circumvallate and foliate papillae[45] and Minor lingual salivary glands.[45]
4. Mesodermal origin: From the paraxial mesoderm derivatives like tongue muscles[52] and endothelial cells,[47,48] and the hematopoietic stem cells, myeloid cells and lymphoid cells.[53]
5. Expression in multiple tissues: Markers such as cytokines, enzymes, collagen and fibronectin have multiple tissue structures that can express these markers.

Based on the site of expression in a cell, the markers expressed in various tissues/cell lineages were broadly classified as.

1. Intracellular markers and 2. Extracellular markers. The intracellular markers mainly constitute

A. The cell surface markers, constituting the Surface receptors, Cell membrane and vesicular transport markers, Cell adhesion markers.
B. The protoplasmic markers are subcategorized into the Cytoplasmic and nuclear markers.

The ECM markers[54,55] constitute

A. The ground substance markers and
B. The Basement membrane markers.

Thus, from our wide review, we have attempted to classify the IHC Marker studied in oral diseases based on the tissue, cell and their site of expression in the cell, mapping to their embryological origin. This classification was made for an easier, better understanding and remembrance of IHC markers for a beginner [Figures 2 and 3].

In the present review, the following facts were observed:

1. The site of expression of IHC markers can be cellular or extracellular. In cellular, it can be expressed in the nucleus, cytoplasm or Cell membrane or it could be in a combination of these sites. Example: p53 expressed in the nucleus,[31] CK 20 in the cytoplasm,[9] CEA in cell membrane,[6] S100A16 in cytoplasm and cell membrane[39] and Oct 4 in Cytoplasm and nucleus.[5] In extracellular, it is expressed in the basement membrane or the ground substance. Example: Type IV collagen in basement membrane[42] and HS-GAG expression at the submucosally,[43] that is, ground substance.
2. The site of expression of one particular marker can vary between normal and pathologic tissue. For example, BCL 10 in PSS expression is at nucleus while in tonsil and lymph nodes, it expresses at cytoplasm,[33] OSMF expression of E-cadherin is at CM and C but in a normal mucosa, it is expressed in Cytoplasm[7] (pAMPK), and (pACC) in pathology is expressed in the cytoplasm and in normal mucosa, it is expressed in the nucleus.[35]
3. Few IHC markers are expressed only in pathology and do not show positivity in normal tissue. For example, CEA shows cell membrane expression in Oral epithelial dysplasia, and OSCC but shows negative expression in normal mucosa.[56] Osteopontin (OPN) shows cytoplasmic expression in OSCC but is faintly detected in the epithelium of oral mucosa or gingiva.[38]
4. Expression of one particular marker by tissues of multiple cell lineages. For example, inflammatory markers having their cell lineages from hematopoietic stem cells are found to be infiltrated in structures of various tissues of origin.[46]
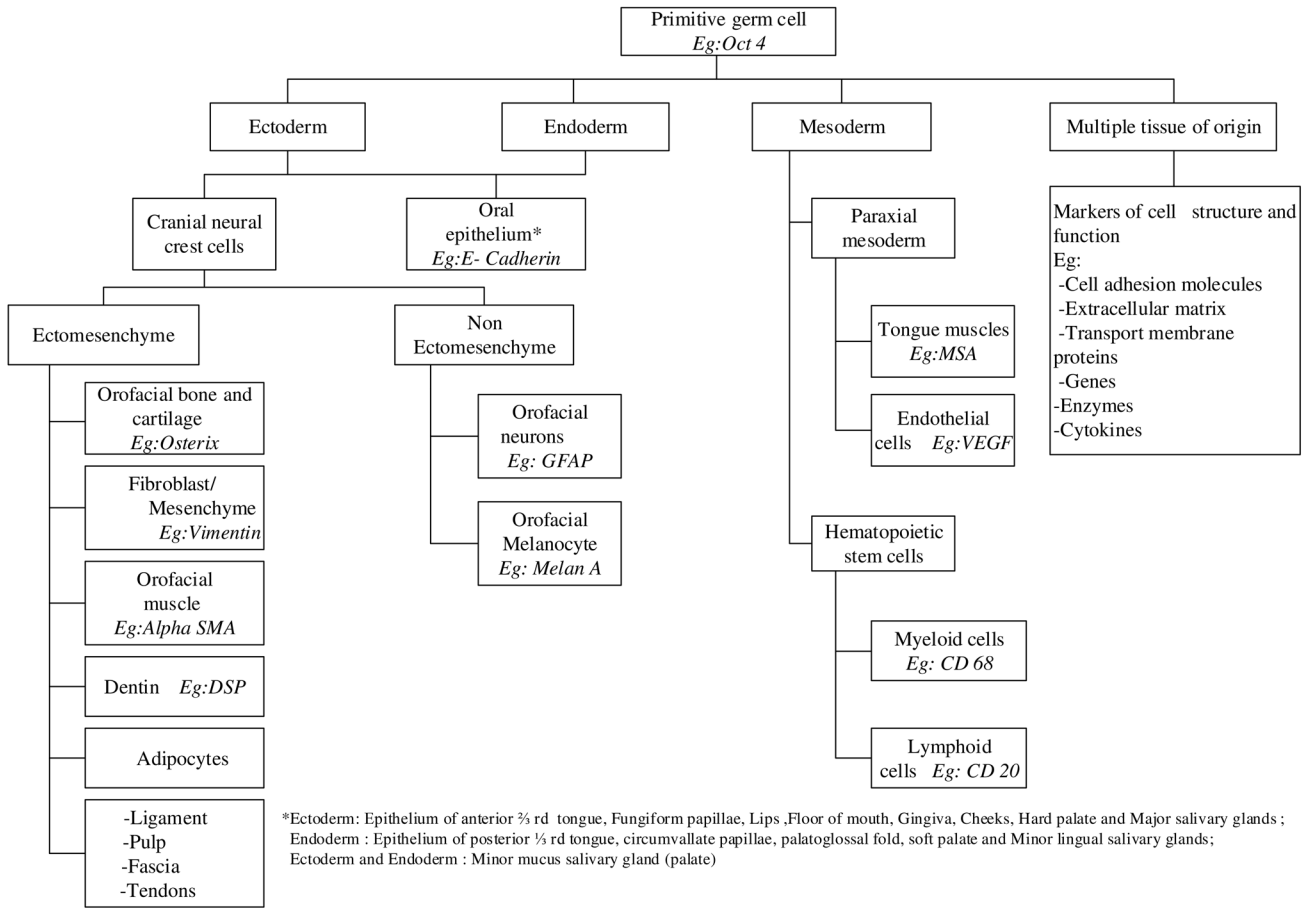5. A tumour of one particular tissue of origin could be positive for markers of other tissue of the same

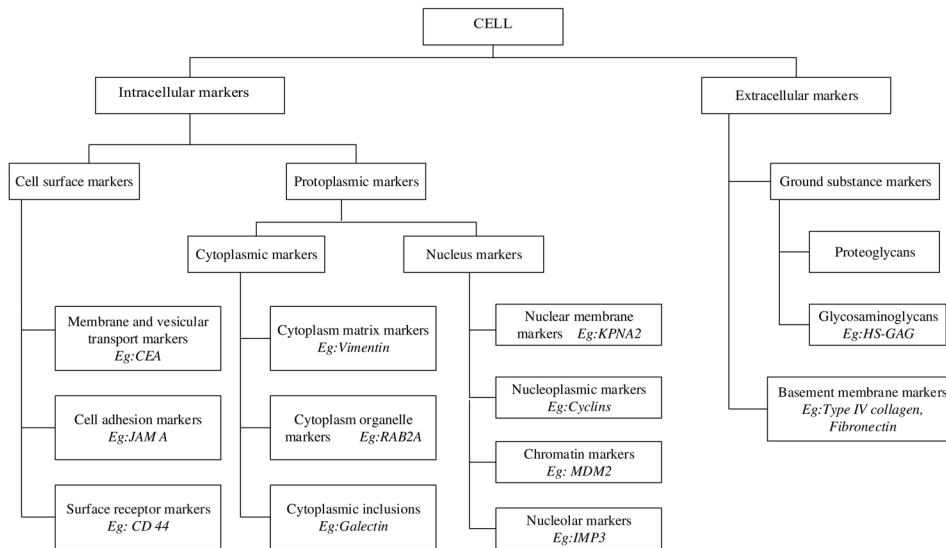**Figure 2:** Classification of IHC markers of oral diseases – an embryological outline

Figure 3: Classification of IHC markers based on the site of expression in a cell

or different cell lineage. For example, OSCC – an epithelial neoplasm of ectodermal origin shows positivity for vimentin.[7] Vimentin is expressed by mesenchymal tissue of the oral cavity. The source of mesenchymal cells in the head and neck is ectoderm whereas in the trunk, it is from mesoderm.

6. A marker may be expressed by different types of cells. For example, Vimentin is expressed by squamous

epithelial cells of OSCC,[7] tumour cells of PLGA[16] and connective tissue cells of the buccal mucosa.[7]

7. Many IHC markers can be positive for a particular pathology. For example, S100,[39] Osteopontin[38] positive in OSCC.

Our review is the first of its kind to classify and enumerate the expression of oral biomarkers based on tissue, cell and site of expression in a cell with the comparison between pathology and normal tissue. This classification can serve as a beginner's guide during IHC interpretation. Since it is a scoping review, it does not provide a complete list of oral biomarkers. Our classification and categorization of markers under tissue, cell and its site of expression in a cell is based on its expression in normal tissue and not based on the pathology in which it is expressed. In the future, a complete list of oral biomarkers with their expression in pathology and normal tissue, specific to tissue, cell and site of expression in a cell can be compiled.

CONCLUSION

In this scoping review, we have derived a classification of IHC markers of oral diseases based on the tissue, cell and site of expression, mapping to their embryological origin. This classification can aid a beginner in the selection, prompt application and interpretation of the IHC markers during diagnosis and research. Thus, we conclude that the IHC markers expressed in various tissues are either directly or indirectly linked with the embryological development of that particular tissue. Therefore, a thorough knowledge of embryology is imperative to fully comprehend the various concepts in IHC.

Authors contribution

All the authors contributed to the study's conception and design. Data collection and analysis were performed by the first, second, third, fourth, fifth, and sixth authors. The first draft of the manuscript was written by the first, second, and third author and all the others commented on the previous version of the manuscript. All the authors read and approved the final manuscript. All the authors agree to be accountable for all the aspects of the work in ensuring the questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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Conflicts of interest
There are no conflicts of interest.

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