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Expression of Ubiquitin C-Terminal Hydrolase-L1 in Minor Normal Salivary Gland Tissues in Perilesional Area to OSCC

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Abstract

Ubiquitin C-terminal hydrolase L1 (UCH-L1), is a deubiquitinating enzyme. Certain changes in ubiquitination attribute to uncontrolled growth, leading to oncogenesis. The aim of this study was to characterize UCH-L1 expression patterns in normal looking human minor salivary gland tissues in the perilesional area of oral squamous cell carcinoma (OSCC). A retrospective study in which UCH-L1 immunohistochemical staining performed for 27 formalin fixed paraffin embedded blocks of surgically removed primary OSCC containing perilesional minor salivary gland tissues. The expression, subcellular localization, pattern of distribution, and stain intensity were assessed. UCH-L1 was immunopositive in minor salivary gland tissues of all sections. The immunoreactivity was (70.4%) detected in the cytoplasm and (29.6%) as mixed expression (cytoplasm and nucleus). UCH-L1 was predominately expressed in the ductal system (40.7%), followed by the myoepithelial cells (33.35%), while it was weakly expressed in the acinar cells; mucous (14.9%) and serous acini (11.1%). UCH-L1 expression is detectable in minor salivary glands in a perilesional area of OSCC, Although previously approved that it was absolutely absent in normal salivary gland tissues, it may activate during the course of oncogenesis and can be classified as a marker for predicting the future invasion of OSCC to adjacent minor salivary gland tissues. This study provides evidence for additional studies on the UCH-L1 expression in normal salivary glands in patients with maxillofacial tumorigenesis.

Introduction:

Salivary glands are exocrine organs, histologically compose from duct-acinar units. They consist of different types of cells: ductal, acinar, and myoepithelial cells. Both ductal and acinar cells located on the luminal side of the duct system, so they called luminal cells. While myoepithelial cells present on the

basement membrane side surrounding the luminal cells [1,2]. Salivary glands tumor comprises less than 3% of all head and neck tumors [3] and display astonishing morphological variations that have considerable diagnostic challenges [4]. The ubiquitin-proteasome system (UPS) plays a significant role in the regulation of almost all aspects of cell cycle including proliferation, differentiation, signaling and apoptosis and antigen presentation [5,6]. UPS function depends on the availability of free ubiquitin [7]. "Ubiquitination is an important post-translational process for the proteasome-mediated degradation of short-lived, abnormally folded, or damaged proteins in eukaryotic cells" [6]. Ubiquitination and deubiquitination processes, which determine the intracellular level of free ubiquitin deubiquitination, carried out via deubiquitinating enzymes (DUBs). Ubiquitin C-terminal hydrolase L1 (UCH-L1), also known as protein gene product 9.5 (PGP9.5), is a deubiquitinating enzyme [7]. Certain changes in Ubiquitination attribute to uncontrolled growth, leading to oncogenesis [8]. In normal tissues, UCH-L1 is exclusively expressed in neural and neuroendocrine cells [9], and low levels present in kidneys, breast epithelium, and reproductive tissues, it is absent in most other tissues [10]. UCH-L1 have been identified in several types of malignant tissues [7,8]. As UCH-L1 activity is implicated in the control of the levels of free ubiquitin and the UPS, the relation of UCH-L1 expression to tumorigenesis has been established [7]. Certainly, increased UCH-L1 level is considered as an early diagnostic marker for some type of malignancies such as colorectal carcinomas, non-small cell lung cancer, breast cancer, pancreatic cancer, oesophageal SCC, gastric cancer, medullary thyroid, prostate, and renal cancer cells [7,8,10]. The previous published literature did not report UCH-L1 expression in normal salivary gland tissues [11,12]. However, tissues adjacent to a malignant growth are not considered to be biologically normal, since perilesional molecular changes were reported to affects the nearby structures including the blood vessels, the fibroblast cell and even normal looking surface epithelium [13]. These observations guide us to hypothesise that an abnormal expression of UCH-L1 might occur as an early molecular alteration that affects the biological activity of cells of minor salivary glands adjacent to the most frequent oral carcinoma. Thus the aim of this study is to characterize the expression pattern, subcellular localization, and stain intensity of UCH-L1 in normal minor salivary gland tissues adjacent to surgically excised OSCC, which may closely relevant to the therapeutic plan and prognosis.

Materials and methods:

The study conducted in University of Sulaimani /School of Dentistry / Department of oral pathology in April 2017. The local ethics committee of the Faculty of Medical Sciences approved this study. The study samples compose of 27 retrospective formalin fixed paraffin embedded blocks of primary OSCC which contain normal perilesional tissue of minor salivary glands. Cases were excluded if the patient known to receive the previous radiotherapy or chemotherapy. Immunohistochemical study was carried out, 5µm sections were cut from each block and performed according to standard techniques. Firstly, the sections were deparaffinized in xylene and rehydrated through graded ethanol. Retrieving of antigen through boiling in citrate buffer (pH-6, 15min). Sections were washed twice with cold phosphate buffered saline (PBS) (3 min for each) at room temperature. By hydrogen peroxidase (10 min), the endogenous activity of peroxidase was blocked. Incubation of sections with primary antibody (diluted rabbit polyclonal anti-UCHL-1, 1:200, Abcam) for 45 min and 4 times washed with PBS. Then they incubated with complement for (10 min) and by PBS washed (3min). Goat anti-rabbit HRP conjugate for 15 min was applied and then washed. Staining of the sections by DAB substrate kit (Vector, Burlingame, CA), for 5 min, and for (20 sec) counter-stained

with hematoxylin. Then they cleared after they dehydrated, and with DPX mounted on slid, to be ready for microscopical examination. Normal nervous tissue found in specimens provide an internal positive control [14]. Immunoreactivity was assessed for the different components of normal minor salivary gland tissues that present in specimens including the acini both serous and mucous, ductal system as intercalated, striated and excretory ducts, in addition to myoepithelial cells. Two observers, without knowledge of patient clinical data, evaluate the immunoreactivity independently and semi-quantitatively. The stain expression assessed in the following parameters included cell types, and subcellular localization: membranous, cytoplasm alone or mixed (both cytoplasm and nucleus), stain intensity assist as weak, moderate, strong [15], also the pattern of stain distribution studied as strong homogenous density and reduced homogenous density. Statistical analysis was done by using SPSS Version 22.0 software and data analyzed by Chi-Square test. P-value <0.05 was considered statistically significant.

Results:

The internal positive control (nerves presented in the prepared tissue section) showed high UCH-L1 expression with strong diffuse homogenous staining Fig.(1). In minor salivary glands, UCH-L1 was immune-positive in all studied sections. The immunoreactivity was mostly cytoplasmic (70.4%). Less frequently seen as mixed expression (29.6%) Table(1). UCH-L1 was predominately expressed in the ductal system (40.7%). It was seen in duct luminal epithelium of all duct systems Fig. (2 and 3), whether they were within intercalated or striated or excretory ducts. Besides, its high expression in myoepithelial cells (33.35%) Fig.(4). However, UCH-L1 was weakly expressed in the acinar cells both mucous (14.9%) and serous (11.1%) acini Fig.(5,6). Regarding the stain intensity, all myoepithelial cells and most of the duct luminal epithelial cells have prominent strong stain intensity (63%) which have strong homogenous distribution (66.7%), while the serous and mucous cells express a weak stain intensity (33.3%) with always reduced homogenous distribution (33.3%) Table (1). The percentages of subcellular expression of UCH-L1 in relation to the intensity of the stain are illustrated in Table (2). The positive mixed UCH-LI expression always appear with strong stain intensity (100%). While UCH-L1 cytoplasmic expression showed the equal percentage of either strong or weak stain intensity (47.4%), there was significant association between subcellular expression of UCH-L1 and the intensity of the stain (p value is 0.035). Regarding the subcellular expression of UCH-L1 in relation to the pattern of expression, the mixed expression of UCH-L1 had a prominent strong diffuse homogenous distribution of stain (100%). On other hand, the cytoplasmic positivity either seen with strong (52.6%) or reduced diffuse homogenous distribution (47.4%), the subcellular expression in relation to the pattern was statistically significant ($p<0.05$). Lastly, the subcellular expression of UCH-L1 in relation to cell type showed that 75% of the duct luminal epithelial cells represent mixed localization. The cytoplasmic expression of UCH-L1 was 36.8% in myoepithelial cells and 26.3% in duct luminal epithelium, Table (2), but the difference was not significant ($P>0.05$).

Discussion:

Although hematoxylin-eosin staining is the main slandered method used in the diagnosis, immunohistochemistry can enhance the accuracy and act as a tool in cases to investigate subjects that cannot be evaluated by histological examination, like cell nature and its state of differentiation, cell proliferation, and expression of tumor protein [4]. Regarding the cell differentiation, the important role of immunohistochemistry for the differential diagnosis of tumors of the salivary gland was to distinguish whether the neoplastic myoepithelial cells are contributing to the

tumor or not [16-18]. Myoepithelioma or myoepithelial carcinoma do not show luminal cell differentiation. The tumors that show acinar cell differentiation and do not differentiate into myoepithelial cells, were considered to be acinic cell carcinoma [4]. Although tumors of the salivary gland exhibit diverse histological features, "they still show differentiation toward the cells that morphologically constitute the normal salivary gland" [18].

Although previously approved that UCTH-L1 cannot be demonstrated in normal salivary glands tissues [11].This study is the first attempt to evaluate the immune -reactive expression of UCH-L1 in normal looking perilesional minor salivary gland tissues adjacent to surgically removed OSCC lesions. In this study, the high level of UCH-LI expression was found to be localized immunohistochemically mainly in the luminal cells of the all duct system, which invariably showed a strong homogenous diffuse pattern of staining of strong intensity and it is mostly with mixed expression. Myoepithelial cells also consistently showed a similar pattern but with nearly the same intensity and with more extent to only cytoplasmic expression. This expression diminished at the level of serous and mucous acini and was completely absent in most regions of the sections. This pattern of staining gives an impression to predict future invasion and expect the type of the tumor that may affect the adjacent salivary gland tissues. The preliminary finding of this study needs conformance by further studies.

Conclusions

UCH-L1 immunohistochemical staining was detected in normal looking minor salivary glands adjacent to OSCC growth in perilesional site. Although previously approved that it was absolutely absent in normal salivary gland tissues, it may activate during the course of oncogenesis and can be classified as a marker for predicting the future invasion of OSCC to adjacent minor salivary gland tissues. This study provides evidence for additional studies on the UCH-L1 expression in normal salivary glands in patients with maxillofacial tumorigenesis.

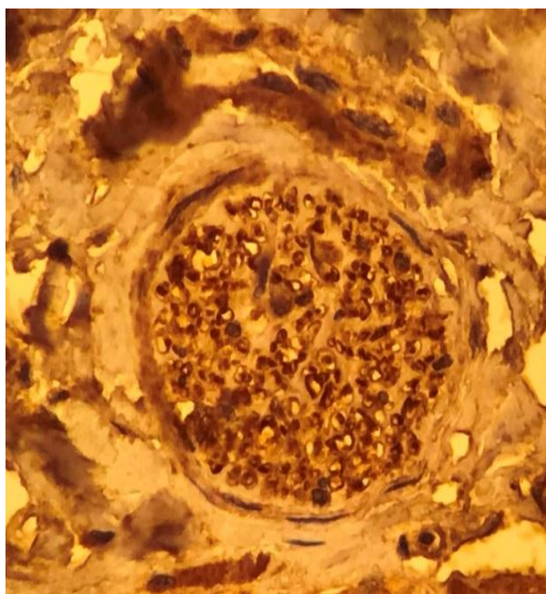


Fig. (1): Expression of UCH-L1 in nervous tissue× 40

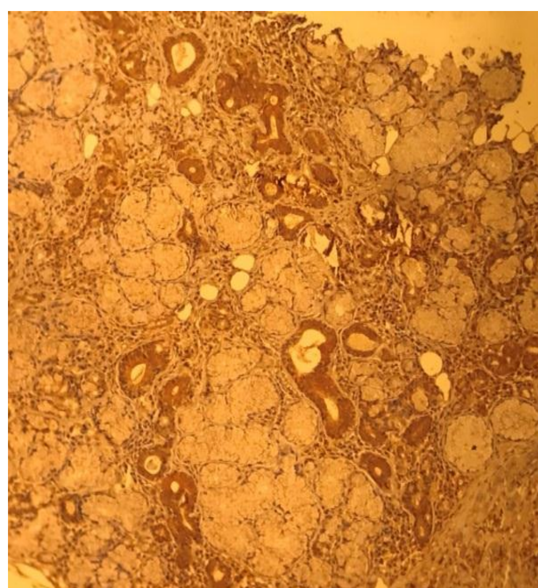


Fig. (2): Expression of UCH-L1 in Duct System×4

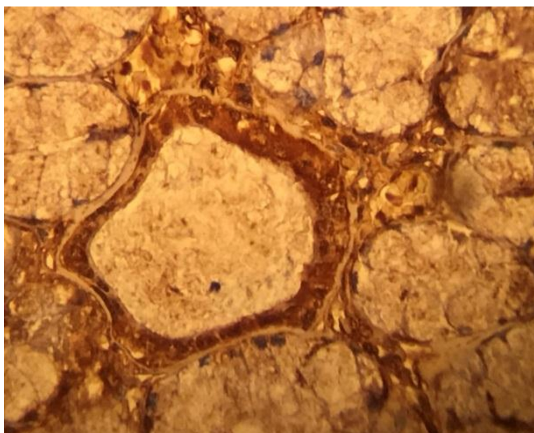


Fig (3): Strong stain intensity of UCH-lL1 in striated duct ×40

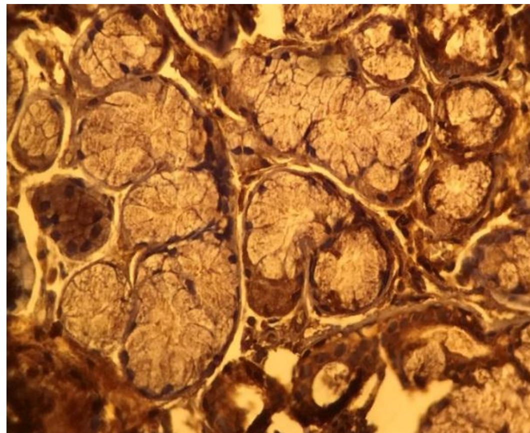


Fig (4): Prominent stain intensity of UCH-lL1 in myoepithelial cells×10

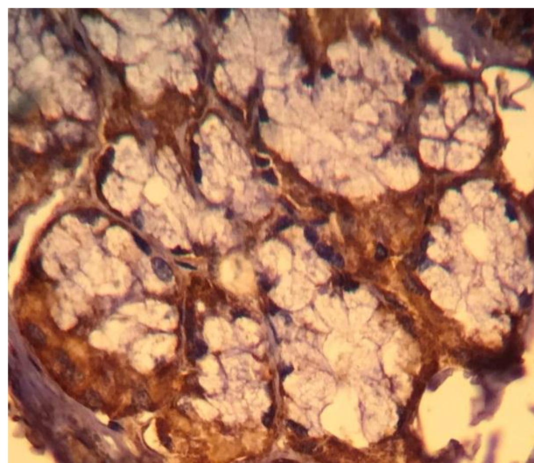
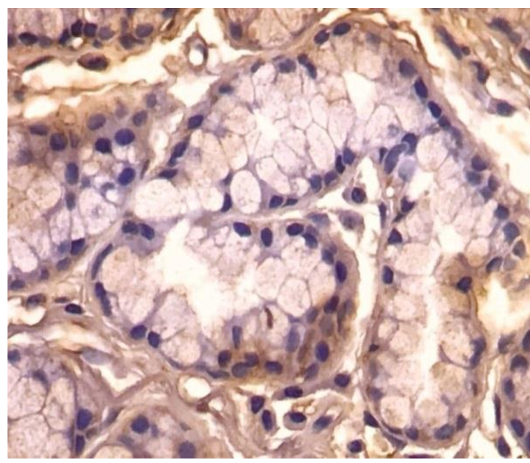


Fig (5): Stain expression seen only in myoepithelial cells ×10.



Fig(6): UCH-L1 Expression seen in myoepithelial cells with weak serous acini involvement ×40.

Table (1): Frequency distribution of UCH-L1 expression in minor salivary gland tissues adjacent to OSCC

		No.	%
Expression	Mixed	8	29.6
	Cytoplasmic	19	70.4
Type of cell	Duct luminal epithelium	11	40.7
	Myoepithelium	9	33.3
	Mucous acini	4	14.9
	Serous acini	3	11.1
Intensity	Weak	9	33.3
	Moderate	1	3.7
	Strong	17	63
Pattern	Strong diffuse homogenous	18	66.7
	Reduced diffuse homogenous	9	33.3

Table (2): Subcellular UCH-L1 positive expression in minor salivary gland in relation to stain intensity, pattern of expression and cell types

		Mixed		Cytoplasmic		P- value
		No.	%	No.	%	
Intensity	Weak	0	0	9	47.4	0.035
	Moderate	0	0	1	5.2	
	Strong	8	100	9	47.4	
Pattern	Strong diffuse homogenous	8	100	10	52.6	0.02
	Reduced diffuse homogenous	0	0	9	47.4	
Cell types	Duct luminal epithelium	6	75	5	26.3	0.091
	Myoepithelium	2	25	7	36.8	
	Mucous acini	0	0	4	21.1	
	Serous Acini	0	0	3	15.8	

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