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IMMUNOHISTOCHEMICAL DISTRIBUTION OF S-100 PROTEIN AND CYTOSKELETAL PROTEINS (A-ACTIN, DESMIN AND VIMENTIN) IN BOVINE TONGUE AND SOFT PALATE

Amin Hassanin[1], Elnasharty Mohamed [2] and Galal A.E. Youssef [3]

[1] Department of Cytology and Histology, Faculty of Vet. Med., Kafr El-Sheikh University, Kafr El-Sheikh, Egypt.

[2] Department of Cytology and Histology, Faculty of Vet. Med., Alex. University, Damanhur Branch, Al-Bostan, Damanhur, Egypt.

[3] Department of Anatomy and Embryology, Faculty of Vet. Med., Mansoura University, Mansoura, Egypt.

ABSTRACT

Distribution and localization of S-100 protein and cytoskeletal proteins, α-actin, desmin and vimentin, in the bovine tongue and soft palate were investigated using immunohistochemistry. S-100 protein was expressed in the taste buds and connective tissue core of circumvallate papillae, moderate immunoreactivity were occur in subepithelial connective tissue cells of the tongue and soft palate. The serous secreting cells and ducts cells in the lingual salivary gland, as well as serous demilunes of mixed palatine salivary glands were strongly immunopositive for S-100 protein. Mucous cells the palatine salivary glands were constantly negative for all tested proteins. Positive staining for α–actin and desmin were observed in the myoepithelial cells, smooth muscle fibers of blood vessels in addition to intrinsic skeletal muscles of tongue and soft palate. Desmin was

expressed in stratum granulosum of keratinized epithelium and supranuclear region of lingual salivary glands. Vimentin was always present in connective tissue cells and nerve bundles in both tongue and soft palate. These results revealed a tissue specific distribution of the tested proteins in bovine tongue and soft palate; that highlight for the use of these proteins as markers for many morphological and pathological aspects.

INTRODUCTION

S-100 protein is classified into a group of calcium binding proteins ***(Isobe et al., 1982; Kahn et al, 1991)*** with a low molecular weight of about 10-24 kDa ***(Fano et al., 1995)*** that are important in intracellular calcium metabolism ***(McNutt, 1998)***. S-100 protein was thought to regulate diverse groups of cellular functions including cell growth, energy metabolism, contraction and intracellular signal transduction ***(Zimmer et al., 1995)***. It was first isolated in the brain and it has long been considered as specific to the nervous system ***(Bock, 1978)***. However, subsequent studies revealed presence of S-100 protein in other tissues as human testis ***(Haimoto et al., 1987)***, human epidermis ***(Rowden et al., 1985)***, bovine testis ***(Amselgruber et al., 1992; Cruzana et al., 2003)*** and a wide variety of organs and tissues including lymph nodes, spleen, thymus, pancreas, salivary and mammary glands ***(Nakajima et al., 1982; Turusov, 1990; Momotani et al., 1993; Nagasao et al., 2002)***. Antibodies against S-100 are widely used in diagnosis of human tumors ***(Turusov, 1990)*** and have been shown to be a useful marker for the epidermal Langerhans cells ***(Takahashi and***

Nakano, 1989). Cytoskeletal proteins desmin and vimentin are intermediate filaments (about 10 nm in diameter) and microfilaments α-actin (diameter up to 7 nm) are normal constituents in the cells but they have a high degree of cell lineage specificity ***(Weber and Osborn, 1982; Weber and Geisler, 1984)***. α-actin is a contractile protein present in muscle and some non-muscular tissues ***(Skalli et al., 1986)*** and detected in myoepithelial cells ***(Zedda and Farina, 1998)***. Desmin is normally present in muscular tissues (skeletal, smooth and cardiac muscles) and its expression is highly specific for muscle and their tumors ***(Azumi et al., 1988)***. Vimentin is present in most of mesenchymal cells ***(Azumi and Battifora, 1987),*** it was localized in epidermal fibroblasts in human skin ***(Rappersberger et al., 1990)*** and expressed in smooth muscles of blood vessels ***(Gabbiani et al., 1981)***.

Little information about the expression of S-100 protein and cytoskeleton proteins in bovine tissues are available. The present study describes the exact localization and differential distribution of S-100 protein and cytoskeletal proteins (α-actin, desmin and vimentin) in bovine tongue and soft palate.

MATERIALS AND METHODS

Sampling:

Specimens from the tongue and soft palate were obtained from 15 healthy adult cattle of both sexes immediately after slaughtering. It washed with phosphate buffer solution (PBS) then fixed in Bouin's solution for 18 hours.

Preparation of paraffin sections:

After fixation, specimens were routinely dehydrated in ethanol and embedded in paraffin for sectioning. By using of rotary microtome, 5-7μm thickness sections were obtained and mounted on poly-*L*-lysine coated slides.

Immunohistochemistry:

Table (1): Antibodies that used in the current study are shown with their dilution.

Antibodies	Name	Type	Code	Source	Dilution
Primary	Rabbit anti S-100	Polyclonal	Z0311	Dako	1:600
	Mouse anti α-actin	Monoclonal	M0851	Dako	1:200
	Mouse anti-desmin	Monoclonal	M0760	Dako	1:50
	Mouse anti-vimentin	Monoclonal	M7020	Dako	1:200
Secondary	Biotinylated goat anti-rabbit	Polyclonal	BA-1000	Vector	1:200
	Biotinylated goat anti-mouse	Polyclonal	BA-9200	Vector	1:200

Procedure:

Tissue sections were deparaffinized and rehydrated for immunostaining using the avidin-biotin peroxidase complex (ABC) method ***(Hsu et al., 1981)***. Sections were pretreated with 3% H_2O_2 in methanol for 15 minutes to block the endogenous peroxidase activity followed by three times washing with PBS, then incubated with 2% normal goat serum for 30 minutes to block the unspecific binding sites. The primary antibodies against S-100 protein, α-actin, desmin and vimentin were diluted in PBS (Table 1) and applied on tissue sections

then incubated at 4˚C in moisture chamber overnight. After three times washing with PBS the biotinylated secondary antibodies were applied (Table 1) for 30 minutes at room temperature followed by three times washing with PBS.

ABC (Avidin Biotin peroxidase Complex, K0355 Dako) was diluted (1ml PBS + 10 µl A + 10 µl B) then applied on sections for 1 hour at room temperature, then rinsed by PBS. Immunostaining was visualized by 3, 3-diaminobenzidine (DAB) and H_2O_2 in Tris buffer within 10 min at room temperature. Sections were counter stained with Mayer's Hematoxylin for 10 seconds, mounted in DPX and examined under light microscope to analyze the results and to make pictures. Control slides were prepared using the same method omitting either primary or secondary antibodies.

RESULTS

Immunohistochemical staining of bovine tongue and soft palate showed a great diversity in tissue distribution of the tested proteins. The results were summarized in the Table 2 & 3.

A. Tongue:

Epithelium lining: The stratum granulosum of the stratified squamous keratinized epithelium was immunopositive with desmin in the form of cytoplasmic granulation (Fig. 2a) however it was immunonegative with other tested proteins. S-100 protein was strongly expressed in sensory cells of the taste buds and its unmyelinated nerve fibers in the subepithelial tissue (Fig. 1b).

Subepithelial tissue: Connective tissue core of vallate papilla, subepithelial connective tissue and nerve bundles in submucosa showed positive immunoreactions with S-100 protein (Fig. 1a, c) α-actin (Fig. 1e) but strong immunostaining of subepithelial connective tissue was occurred with vimentin (Fig. 2d).

The lingual glands: Serous secreting cells and duct cells were immunopositive for S-100 protein, which was localized in the nucleus and cytoplasm (Fig.1d) while the supranuclear portion of these cells gave a granular positive reaction for desmin (Fig. 2b). Myoepithelial cells were positively reacted with α-actin (Fig. 1f) and desmin (Fig. 2b insert).

Muscles: Smooth muscle fibers of the blood vessels and intrinsic skeletal muscle showed strong reaction for α-actin (Fig. 1f) and desmin (Fig. 2c).

Connective tissue core: Vimentin was particularly localized in the connective tissue cells in the inter-glandular and inter-muscular connective tissue (Fig. 2e); as well as intralobular connective tissue between the serous acini (Fig. 2f)

B. <u>Soft palate</u>:

Epithelium lining: The stratum granulosum of the stratified squamous keratinized epithelium was weakly immunoreactive with desmin (Fig. 4a) and negative with other tested proteins.

Subepithelial and submucosa: The connective tissue cells were strongly immunopositive with vimentin (Fig.4d), weak with S-100 (Fig. 3a-insert) and negative with α-actin (Fig. 3d) and desmin (Fig. 4a).

Palatine glands: The serous demilunes of the mixed palatine salivary glands was immunopositive with S-100 protein showing strong cytoplasmic and nuclear staining (Fig. 3a, b); however, mucous secreting cells were constantly negative for all tested proteins. On the other hand, myoepithelial cells were positive with α-actin (Fig. 3f) and desmin (Fig. 4c).

Muscles: Smooth muscle fibers of blood vessels as well as skeletal muscles were immunopositive with α-actin (Fig. 3e-insert, d) and desmin (Fig. 4b-insert, c-insert).

Nerve bundles: Nerve bundles in between the muscular and glandular tissues were positive for S-100 protein (Fig. 3c) and vimentin (Fig. 4e).

Adventitious connective tissue: Fibroblast and fat cells in adventitious connective tissue and around glandular ducts were strongly immunopositive with vimentin (Fig. 4f).

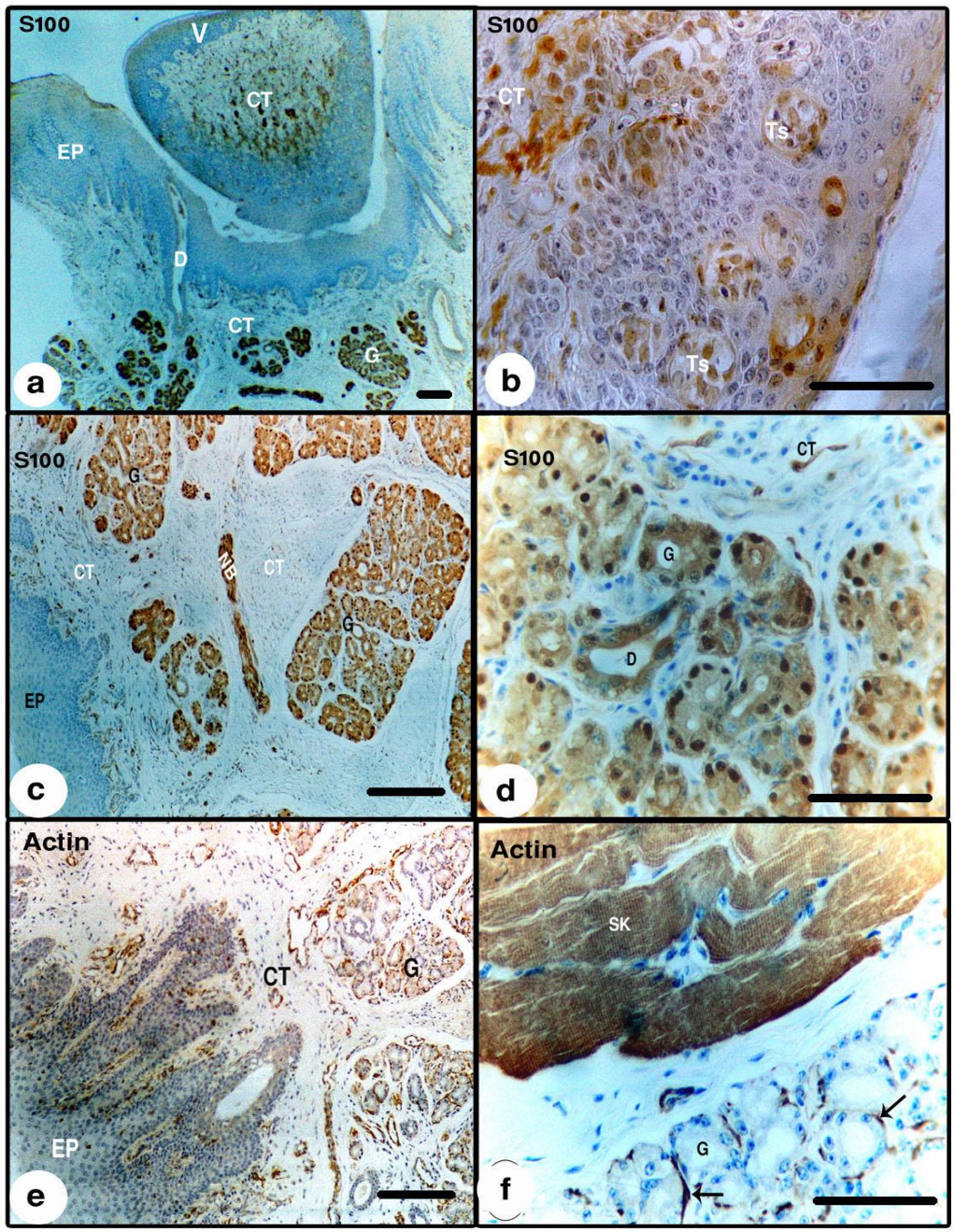
LEGENDS

Figure 1: S-100 was expressed in CT core of vallate papilla (a) taste buds (b) and lingual salivary gland (c, d); α-actin was present in subepithelial CT (e) skeletal muscles and myoepithelial cells (f, f-arrow). CT, connective tissue; G, glands; D, duct; EP, epithelium; NB, nerve bundles; SK, skeletal muscle; TS, taste buds, V, vallate papilla. Bars 40 μm

Figure 2: Desmin was expressed in stratum granulosum of tongue epithelium (a) glandular cells (b) myoepithelial cells (b-insert) and skeletal muscle (c); vimentin was present in subepithelial CT (d) interlobular CT (e) and intralobular CT (f). CT, connective tissue; G, glands; D, duct; EP, epithelium; SK, skeletal muscle. Bars 40 μm

Figure 3: S-100 was expressed in subepithelial CT, dendritic cell (a-insert) and palatine glands of bovine soft palate (a) serous demilunes (b-arrow) and nerve bundles (c); α-actin was present in skeletal muscles (d) smooth muscle of b. vs. (e, e-insert) and myoepithelial cells (f). BV, blood vessel; CT, connective tissue; G, glands; D, duct; EP, epithelium; NB, nerve bundles; SK, skeletal muscle; SM, smooth muscle fibers; TS, taste buds. Bars 40 μm

Figure 4: Desmin was detected in smooth and skeletal muscle (a, b & c-insert) and myoepithelial cells (c); vimentin was localized in subepithelial CT (d) fibroblast cells (d-insert) nerve bundles (e) and adipose tissue (f). BV, blood vessel; CT, connective tissue; G, glands; D, duct; EP, epithelium; My, myoepithelial cells; NB, nerve bundles; SK, skeletal muscle; SM, smooth muscle fibers. Bars 40 μm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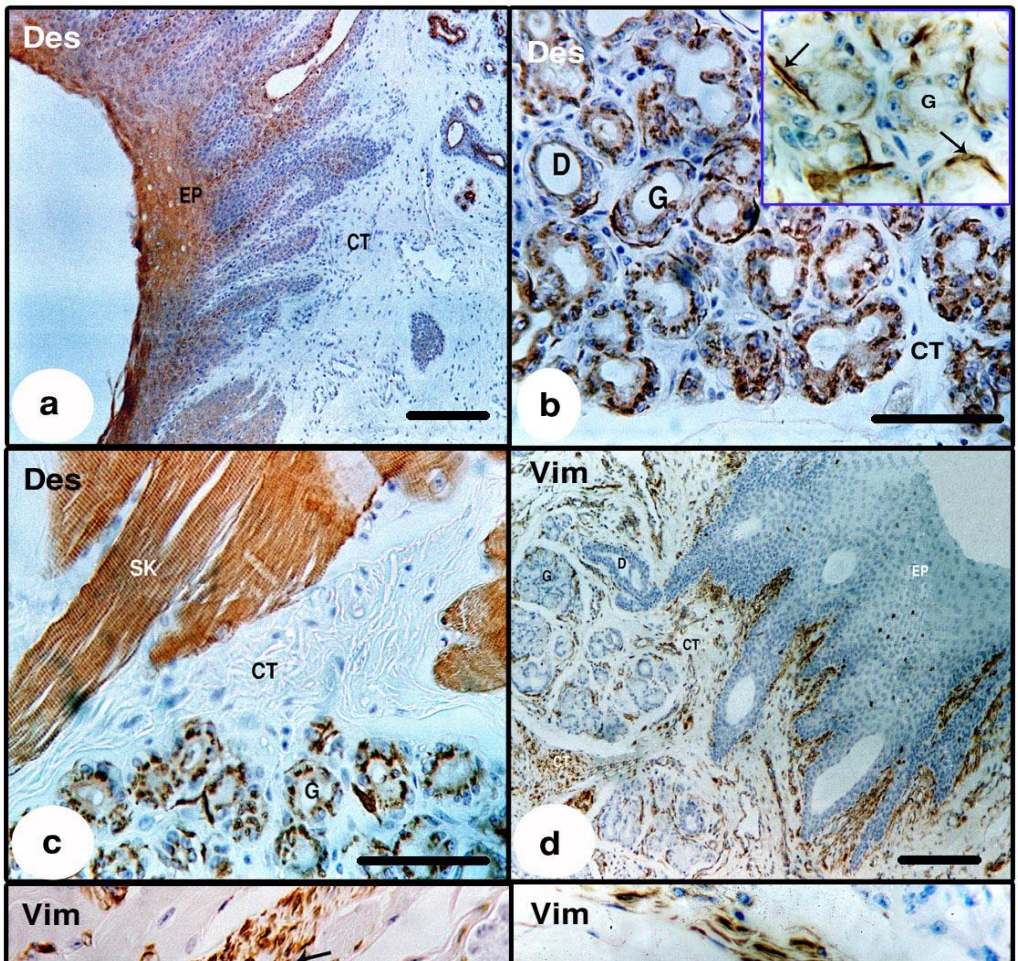
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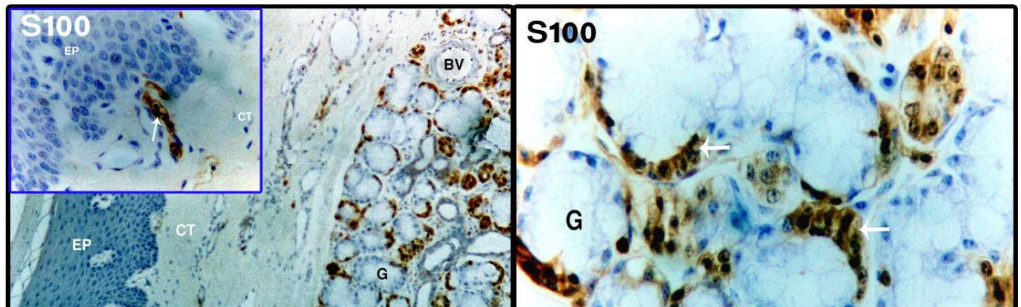
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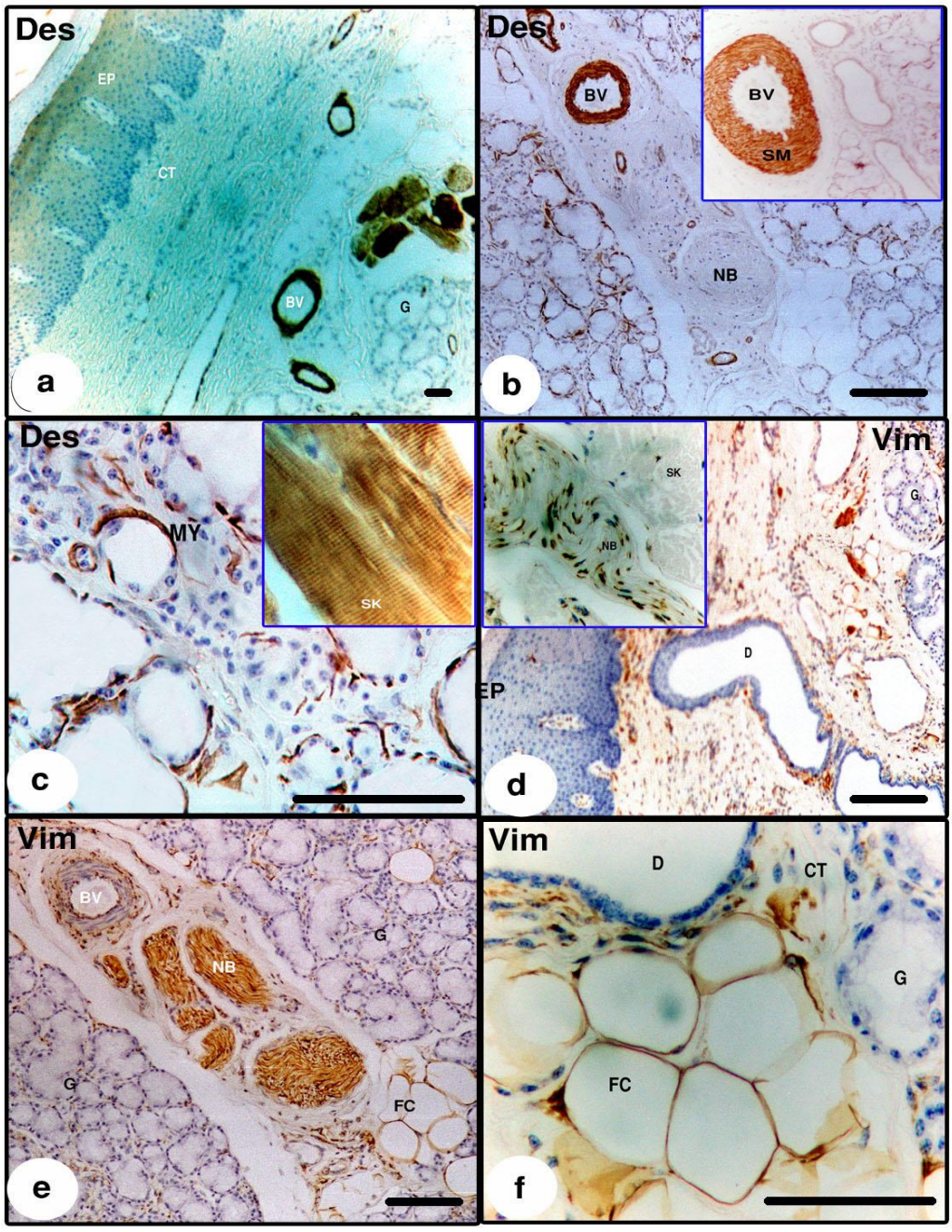
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Table (2): Immunoreactivity of S-100 protein and cytoskeleton proteins in bovine tongue (n=15)

Tongue tissues	S-100 protein	Cytoskeleton proteins		
		α-actin	Desmin	Vimentin
Epithelial	-	-	+**	-
Taste buds	+	-	-	-
Subepithelial c. t. and submucosa	+	+	-	+*
palatine glands				
- Serous cells	+	-	+	-
- Duct cells	+	-	+	-
- Myoepithelial cells	-	+	+	-

Nerve bundles	+*	-	-	+*
Muscles	-	+	+	-
Connective tissue core	-	-	-	+*

***** Strong reaction ****** In stratum granulosum

Table (3): Immunoreactivity of S-100 protein and cytoskeleton proteins in bovine soft palate (n=15)

Soft palate tissues	S-100 protein	Cytoskeleton proteins		
		α-actin	Desmin	Vimentin
Epithelial	-	-	+**	-
Subepithelial connective tissue	+	-	-	+*
palatine glands				
- Serous demilunes	+*	-	-	-
- Myoepithelial cells	-	+	+	-
Nerve bundles	+	-	-	+
Muscles	-	+	+	-
Adventitious connective tissue	-/+	-	-	+*

* Strong reaction ** In stratum granulosum

DISCUSSION

This immunohistochemical study reveals the presence of S-100 protein in the taste buds and its unmyelinated nerve fibers in the underneath connective tissue in the tongue, as well as nerve bundles in the tongue submucosa and soft palate inter-glandular and inter-muscular tissues. This result was confirming the neurologic origin of S-100 as it was first isolated from the bovine brain tissue and identified in glial and Schwann cells **(*Turusov, 1990*)**.

In the present study, the serous secreting and duct cells in lingual salivary glands as well as serous demilunes of mixed palatine glands

were strongly expressed S-100 protein in the form of nuclear and cytoplasmic staining. Similar expression of S-100 was recorded in bovine exocrine glands **(*Lauboeck and Egerbacher, 1997*)** and human salivary glands **(*Nakazato et al, 1985 and Ferraris et al, 2000*)**, in contrary, negative immunoreactivity was found in parotid salivary glands **(*Dardick et al, 1991; Makino et al, 1992 and Lee et al, 1993*).** On the other hand, myoepithelial cells showed negative staining with S-100 protein in lingual and palatine salivary glands. These finding were compatible with the results found in myoepithelial cells in bovine nasolabial glands **(*Zedda and Farina, 1998*)** and human palatine glands **(*Dardick et al, 1991*)**. The immunohistochemical studies of S-100 protein gave conflicting results with the myoepithelial cells of salivary glands, it was recorded as being positively stained as reported by **(*Kahn et al, 1983; Hara et al, 1983; Loeffel et al, 1985; Nakazato et al, 1985; Vanstapel et al, 1986 and Ferraris et al, 2000*)** and negative in other investigation **(*Mori et al, 1987; Ninomiya et al, 1989 and Zedda & Farina, 1998*).** These variations in the staining patterns were contributed to the use of polyclonal or monoclonal antibodies to S-100 protein or its various subunits **(*Molin et al, 1984; Takahashi et al, 1984; Vanstapel et al, 1986 and Ninomiya et al, 1989*)**. Because of this variation of S-100 protein expression in the salivary glands myoepithelial cells, the muscle-type α-actin was considered the more specific markers for myoepithelial cells **(*Gown et al, 1985; Tsukada et al, 1987 and Guglitta et al, 1988*)**. The data represented here showed the myoepithelial cells of lingual and palatine salivary glands were clearly immunopositive for antisera of α-actin and desmin; which indicated the contractile function of myoepithelial cells **(*Ogawa, 2003*).** Smooth muscles of blood vessels as well as skeletal muscle fibers were expressing the microfilament α-actin and the intermediate filaments desmin confirming their ability to

contracts ***(O'Rahilly and Meller, 1994)***. Vascular smooth muscle cells were distinguished by the predominance of muscle-specific alpha type actin; whereas gamma-type smooth muscle actin was present only as a minor component that reflected a differentiation from the other smooth muscle cells **(*Gabbiani et al, 1981*)**. Connective tissue cells, principally fibroblasts, around ducts of salivary glands and adipose tissue were strongly expressing vimentin; these findings were compatible with results obtained in palatine tonsils **(*Koshi et al, 2001*)**. Vimentin was expressed in many cells of mesodermal origin ***(Azumi and Battifora, 1987)***. Unlike the other tested proteins, desmin was expressed in the stratum granulosum of the keratinized epithelium in tongue and soft palate; these findings need further studies to be clarified.

In conclusion, our results indicates that S-100 protein seems to be a multifunctional protein because it is expressed in migratory dendritic cells and secretory glandular cells beside its specific pattern of distribution; together with the cytoskeletal proteins α-actin, desmin and vimentin in tongue and soft palate could be used as biomarkers in bovine salivary glands, muscular and connective tissues.

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