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Comparative Histological and Histochemical Inter-Species Investigation of Mammalian Sub Mandibular Salivary Glands

[1]F. Yazadni Moghaddam, [2]J. Darvish, [2]N. Mahdavi Shahri,
[3]A.S. Abdulamir, [2]M. Mousavi and [4]Siti Khalija Daud
[1]Faculty of Sciences, University of Ferdowsi, Mashhad, Iran
[2]Rodents Group, University of Ferdowsi, Mashhad, Iran
[3]Faculty of Medicine, Alnahrain University, Iraq
[4]Faculty of Science, University Putra Malaysia, Malaysia

Abstract: The major and accessory sub mandibular glands were obtained from different species of rodents belong to families Muridae (*Meriones lybicus*, *Mus musculs*, *Cricetulus migraturus*, *Nesokia indica*, Laboratory hamster and *Apodemus* sp.)? Dipodidae (*Alactage elater* and *Jucullus blanfordi*) and Sciuridae (*Funambulus pennanti*). The skulls of these species were separated immediately after death and fixed in buin with decalcification. Five um sections were stained with hematoxilen-eosin and tetrachrom. Periodic acid shiff and alcian blue (pH 1) were performed for sulfated natural mucins. Microscopic histological features, including existence of mucus and serous acini, presence of different kinds of tubules and different types of ducts as well as the histochemical characteristic features including histochemistry of different tubules and ducts in 2 different pH levels in the major and accessory sub mandibular glands in different rodents showed that the studied species proved to be different and the histological and histochemical study of sub mandibular salivary glands proved practicable with good discriminatory potential in evaluating the inter-species differences.

Key words: Mucin, rodents, sub mandibular, salivary, histochemistery, histology, inter-species

INTRODUCTION

Salivary glands have an important role in terrestrial animals, provide lubrication for eating and vocalisation, aide digestion and supply saliva for pH buffering (Jaskoll *et al*., 2002). Salivary glands of rodents are important elements regarding their adaptations to different diets, environments and taxonomic studies (Stimson *et al*., 2007; Yamada *et al*., 2006). To reach a delicate analysis between biology and ecology of rodents, there is need to study salivary glands histologically and histochemically. The main salivary glands are 3 pairs: parotid, submandibular and subligual glands (Buchalczyk, 1991; Kimura *et al*., 1998). Salivary glands are of importance in research because of their different functions (Asari *et al*., 2000). Much information about their activities, like the secretion type, physiologic functions of the secreted substances, mechanism of water transfer, electrolytes and nerve receptors of salivary glands have been obtained in different animals (Junqueira, 1967; Mese and Matsuo, 2007; Harrison *et al*., 2001; Toyoshima and Tandler, 1991).

Salivary glands develop at different sites and they have very different architectures and produce different types of saliva (Jaskoll *et al*., 2002). Salivary glands have very different architecture and produce different types of saliva (Jaskoll *et al*., 2002). Saliva has many essential functions. As the first digestive fluid in the alimentary canal, saliva is secreted in response to food, assisting intake and initiating the digestion of starch and lipids. Variations in salivary flow can be affected, reversibly or irreversibly, by numerous physiological and pathological factors (Mese and Matsuo, 2007).

The submandibular glands in many rodents, the intercalated and striated ducts are separated by the granular ducts. In the mouse the submandibular gland is a mixed gland with both serous and mucous cells. In contrast in the rat, the submandibular gland has mostly mucous cells and produces only small amounts of Amylase. The submandibular is the first major salivary gland to develop in the embryo, followed by the neighbouring sublingual and then the parotid (Tucker, 2007). In the mouse the activity of the 3 glands also differs between males and females. The male sub mandibular gland has around 10 times more activity than the female (Hosoi *et al*., 1978). The submandibular gland shows a classic branching morphogenesis and has been

Corresponding Author: Siti Khalija Daud, Faculty of Science, University Putra Malaysia, Malaysia

used as a model of organ culture *in vitro* for over 50 years. Weight changes of such glands are different through life, so that the weight of sub mandibular glands increases with body weight increase, while the largest parotid glands can be observed in the youngest animals (Tamarin and Sreebng, 1989). Large salivary glands secrete into the oral cavity through one or several extra glandular ducts, but small salivary glands placed in the mucosa or sub mucosa open into the epithelial layer of the mucosa directly or indirectly through abundant short ducts. Mucous secreting units in the salivary glands of the mammals are similar to acinus, demilunes or goblet cells (Tamarin and Sreebng, 1989).

The shape of mucous secreting cells are cubic to cylindrical, their nuclei are ellipse and are compacted towards the base of the cells. Such cells contain the specific character of mucous secreting cells. Mucosal cells are organized in tubular with cylindrical radiuses from secreting cells surrounding a duct (Carmanchahi *et al*., 2000). Adjacent secreting cells have been connected with 3 types of junction complexes: tight conjunctions, desmosomes and gap junctions (Shackelford and Klapper, 1962; Shackelford and Schneyer, 1964). The progress in discerning the structure and function of cells and tissues in health and disease has been achieved to a large extent by the continued development of new reagents for histochemistry, the improvement of existing techniques and new imaging techniques (Zuber *et al*., 2007).

Histochemically, mucosal units react strongly with staining techniques, Alcian Blue (AB) and PAS (Shackelford and Schneyer, 1964). Mucins with acidic properties are of 2 categories: Sialomucins (containing cialic acid) and sulfomucins (containing sulfate group). Mucins lacking detectable acidic properties are called natural mucins (Kimura *et al*., 1998). In this study, histology and histochemistry of sub mandibular salivary glands specimens were studied in laboratory hamster and 8 rodents, namely, *Funambulus pennati* from Scuridae, *Cricetulus migratorius*, *Meriones libycus*, *Mus musculus*, *Nesokia indica* and *Apodemus* sp. from Muridae and 2 specimens from Dipodidae family *Allactaga elater* and *Jaculus blanfordi.*

MATERIALS AND METHODS

The specimens of *Funambulus pennati* from Scuridae, *Cricetulus migratorius*, *Meriones libycus*, *Mus musculus*, *Nesokia indica* and *Apodemus* sp. from Muridae and 2 specimens from Dipodidae family *Allactaga elater* and *Jaculus blanfordi* were collected from different regions of Khorasan (Tandoureh Park, Moghan, Gonabad, Birjand and Kashmar). The whole skull was selected because it is hard to locate definitely the sub mandibular glands. Moreover, histological autopsy may harm the gland tissue.

After washing blood off the heads with normal saline, they were placed in separate labeled buckles containing bouin's fixative. Tissue preparation processes like passaging, microtomy and staining were then done. Staining was done with Hematoxylin-Eosin, tetrachrome, PAS alcian blue (pH = 1) and PAS alcian blue (pH = 2.5). Microscopic studies were done with light microscope. The slides of all species were studied and compared with each other. The sections were photographed with a photomicroscope. Choosing the appropriate qualitative characteristic features is one of the most important parts of such research in order that necessary distinctions can be shown among different species.

Eleven characteristic features (6 histologic and 5 histochemical) were chosen in the sub mandibular salivary glands (Table 1 and 2).

Statistical analysis: Statistical analysis was done by SPSS software. Kruskal wallis and Chi-square (χ^2) tests were done on qualitative data of sub mandibular salivary glands and $p>0.05$ was considered significant.

RESULTS AND DISCUSSION

All Accessory Sub Mandibular Gland (ASMG) and Sub Mandibular Glands (SMG) were located in the posterior end of the tongue (Fig. 1). Distinctive and characteristic features of the histological investigations were found regarding mucus glands, their ducts, serous gland ducts, the presence or absence of serous demilunes at mucous acinis and the position of demilunes on mucous parts.

Serous and mucous acini were found in the majority of sub mandibular glands of *hamster Nesokia indica*, *Cricetulus migratorius*, *Allactaga elater*, *Funambulus pennati*, *Meriones libycus* and *Apodemus.* However, *Jaculus blanfordi* contains only serosa acini. Based on the resulted histochemical characteristics, there were remarkable differences among the studied species. In addition, the histochemistry of acini in serosal glands showed that most of the species possess neutral mucin (Table 1).

Regarding Table 1 there are no mucin in ducts of the major sub mandibular glands of all species. At 2 different pH levels, there was weak acidic and sulfated mucin in acini of different species. In addition, laboratory hamster can be differentiated from other species because of the lack of acidic mucin in mucosal asini (Fig. 2).

Table 1: The histological and histochemical characteristics* of major sub mandibular salivary glands

Species	Histo-chemistry of serous demilunes		Histo-chemistry of ducts		Histo-chemistry of serous acini		Histo-chemistry of mucous acini		Dominance of mucin		Dominance of duct	Type of duct	Dominance of acini	Presence of serous demilunes	Existence of serous acini	Existence of mucous acini
	pH1	pH2.5	pH1	pH2.5	pH1	pH2.5	pH2.5	pH2.5	pH1	pH2.5						
Laboratory hamster	NM	SIM	UN	UN	±R.	UN	UN	UN	NM	-	ED	SD ED ID	Mucous	-	+	+
Mus musculus	NM	SIM	UN	UN	1R.	±R.	2B	2B	NM	SIM	ED	ED SD	Mucous	+	+	+
Nesokia	NM	SIM			absent		1B	1B	NM	SIM	SD	ED SD		-	-	
Indica	SM	SIM	UN	UN	2R.	2R.	2B 2P	2B 2P	SM	SIM	ID	ID SD	Mucous	+	+	+
Apodemus sp.	NM	NM	UN	UN	1R.	2R.	1R.	1R.	NM	NM	ED	ED SD	Mucous	+	+	+
Meriones libycus	SM	SIM	UN	UN	1R.	±R.	2B 1B	2B 1B	SM	SIM	SD	ED SD	Mucous	+	+	+
Allactaga elater	SM	SIM	UN	UN	1R.	±R.	2B 1B	2B 1B	SM	SIM	ED	SD ED	Mucous	+	+	+
Jaculus blanfordi	SM	SIM	UN	UN	±R.	±R.	2B	2B	SM	SIM	ED	SD ED ID	Mucous	+	+	+
Cricetulus migratorius	NM	SIM	UN	UN	1R.	2R.	1B	1B	NM	NM	ED	SD ED	Mucous	-	+	+
Funambulus pennati	NM	SIM	UN	UN	1R.	2R.	1P	1P	SM	SIM	ED	ED SD	Mucous	+	+	+

*: SMG = Submandibular Gland, ASMG = Accessory Submandibular Gland, MA = Mucous Acini, SA = Serous Acini, MF = Muscle Fiber, ED = Excretory Duct, SD = Striated Duct, ID = Intercalated Duct, MT = Mucous Tubule, SD = Serous Demilunes, S = Septum, GT = Convoluted Granular Tubule, B: Blue acidic mucin, R: Red, neutral mucins, P: Purple, UN: Unstained

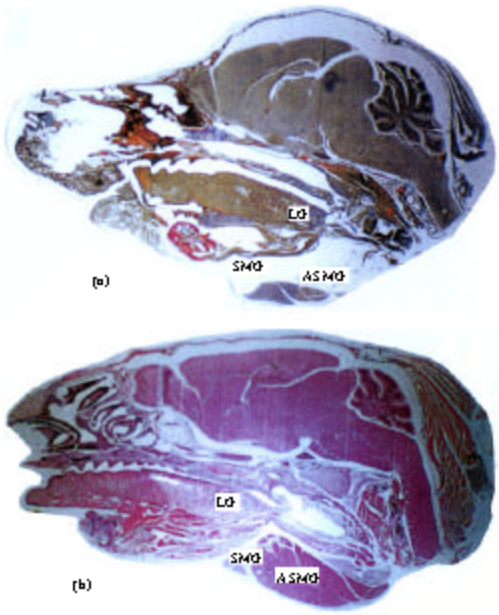

Fig. 1: The location of Accessory Sub Mandibular Glands (ASMG), Sub Mandibular Glands (SMG) and Lingual salivary Glands (LG) in (A) *Allactaga elater* and (B) *Mus musculus*

There were many differences between convoluted granular tubule and acini in major sub mandibular glands of all studied species. Convoluted granular tubules in *Mus musculus* were abundantly present more than serous acini but in *Nesokia indica*, laboratory hamster, *Cricetulus migratorius*, *Funambulus pennati*, *Meriones libycus*. *Allactaga elater*, *Jaculus blanfordi* and *Apodemus* sp., serous acini were higher than convoluted granular tubule.

There were also many differences in the types of ducts and types of dominancy of ducts among the studied species. Neutral mucin acini of *Mus musculus*, *hamster laboratory*, *Nesokia indica*, *Cricetulus migratorius*, *Allactaga elater* and *Jaculus blanfordi* were the highest but *Funambulus pennati*, *Meriones libycus,and Apodemus* sp. were without any neutral mucin (Table 2). All ducts in accessory sub mandibular glands were AB^-, PAS^- (Fig. 3). Histochemical characteristics of acini, convoluted granular tubules and ducts can differentiate between *Meriones libycus* and other species (Table 2).

Accessory sub mandibular glands can also, differentiate *Apodemus* sp. from all other species.

The results of Kroskal walis test on the qualitative data of accessory sub mandibular glands showed that, among the histologic charecterestics, the dominancy of acini versus convoluted tubules and among the histochemical characteristic features, the histochemistry of serosi acini and convulated tubles (based on sulfomucins) were significantly different among the studied species.

In accessory sub mandibular glands, the histochemistry of serosi acini, serous demilunes and the dominancy of mucin based on (sulfomucins sulfate mucins, sialomucins and neutral mucins) were significantly different among the studied families and subfamilies.

In addition, in accessory sub mandibular glands, the chi-square (χ^2) analysis showed that among all qualitative characters and similar to the results of Kruskalwallis test, the dominancy of acini and convoluted tubules and histochemistry of convoluted tubules and serosi acini based on (sulfomucins and acidic mucin and neutral mucins) were different among the studies species. Regarding the histology of major sub mandibular glands, the mocousal acini, from the point of view of existence of neutral mucins and soalomucin, showed a significant difference among the studied species. In addition, the

Table 2: The histological and histochemical characteristics* of the accessory sub mandibular salivary glands

Species	Histochemistry of serous demilunes: pH1	pH2.5	Histo-chemistry of ducts: pH1	pH2.5	Granular tubules: pH1	pH2.5	Histo-chemistry of acini: pH1	pH2.5	Dominance of duct	Type of ducts	Dominant acini or convoluted glandular tubules	Presence of convoluted tubules	Existence of serous acini	Existence of mucous acini
Laboratory hamster	Absent	Absent	UN	UN	UN	±R.	1R.	1R.	GI	GI-SD ID	Acini	-	-	+
Mus musculus	Absent	Absent	UN	UN	UN	UN	1R.	1R.	GI	GI SI	Acini	+	-	+
Meriones indica	Absent	Absent	UN	UN	UN	UN	1R.	1R-1	GI	GI-ID ID	Acini	- +	-	+
Apodemus sp.	Absent	Absent	UN	UN	UN	UN	UN	UN	GI	GI-SD ID	Acini	+	_-	+
Meriones libycus	Absent	Absent	UN	UN	UN	UN	UN	UN	GI	GI-SD ID	Acini	+	-	+
Allactaga elater	Absent	Absent	UN	UN	UN	UN	1R.	1R-1B	GI	GI-SD ID	Acini	+	-	+
Jaculus blanfordi	Absent	Absent	UN	UN	UN	1B-1P	2R-1P	1R.	GI	GI-ID ID	Acini	+	+	+
Cricetulus migratorius	Absent	Absent	UN	UN	1R.	1R.	1R.	1R.	GI	GI-ID ID	Acini	- +	-	+
Funambulus pennati	Absent	Absent	UN	±R.	UN	UN	UN	UN	SD	SD-ID	Acini	+	-	+

*: SMG = Submandibular Gland, ASMG = Accessory Submandibular Gland, MA = Mucous Acini, SA = Serous Acini, MF = Muscle Fiber, ED = Excretory Duct, SD = Striated Duct, ID = Intercalated Duct, MT = Mucous Tubule, SD = Serous Demilunes, S = Septum, GT = Convoluted Granular Tubule, B: Blue acidic mucin, R: Red neutral mucins, P: Purple, UN: Unstained

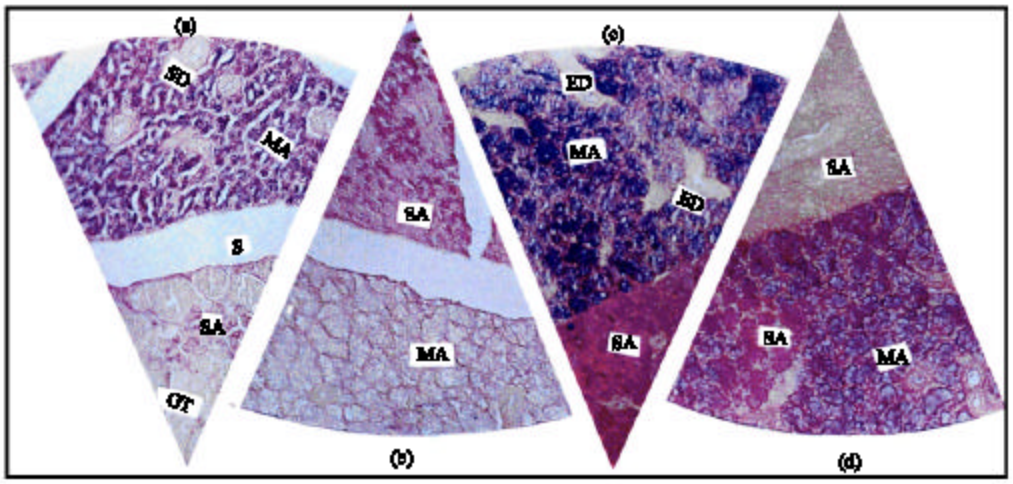

Fig. 2: PAS-Alcian blue (pH1) in major sub mandibular salivary glands of (a) *Mus musculus*, (b) Laboratory hamster, (c) *Allactaga elater* and (d) *Apodemus* sp

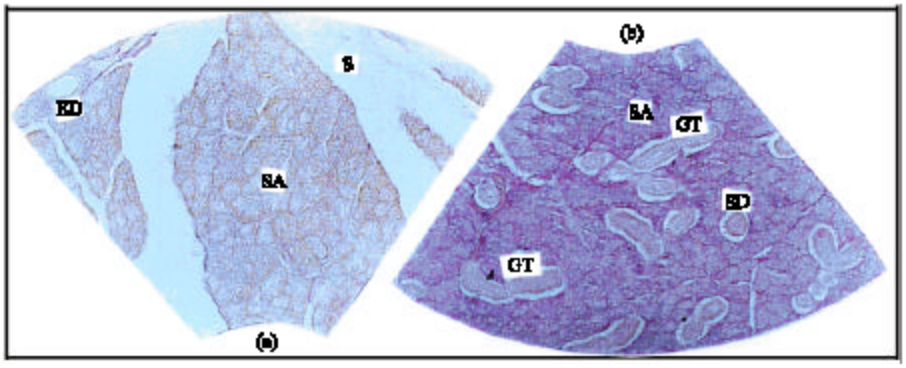

Fig. 3: PAS-Alcian blue (pH1) in accessory sub mandibular salivary glands of (a) *Funambulus pennati* and (b) *Cricetulus migratorius*

histochemistry of serosi acini and mocousal acini and serous demilunes (based on sulfate mucins, sialomucins) in major sub mandibular glands showed also a significant difference among studied groups.

The histological and histochemical features in accessory sub mandibular glands including the dominance of acini and convulated tubles, histochemistry of convulated tubles, the nature of serosi acini based on sulfomucins, acidic mucin and neutral mucins, were all different and proved good discriminating factors among the studied species. In addition, the histological and histochemical features in major sub mandibular glands, including mocousal acini based on existence of neutral mucins and sialomucin, histochemistry of serosi acini, serous demilunes, dominance of mucin versus sulfomucins, sulfate mucins, sialomucins and neutral mucins, were all different among the studied rodents.

Although, there is some variation in the carbohydrate histochemistry of the acini of the accessory sub mandibular glands, these secreting units are classified as seromucous secreting glands. Granular tubules were absent in the major sub mandibular glands and they were also absent in the accessory sub mandibular glands of *Funambulus pennati*.

In the studied specimens, there were some differences in sulfate acidic mucins and sialomucins and in the sections staining intensity of these glands. The rodents species involved in this study were selected carefully to make comparison between diet and different histological and histochemical characteristics. The selected rodents were highly diversified species in terms of dietary habits. Due to the close association of saliva to the environmental diet and climate, it is therefore, practicable to apply comparitive inter-species study based on the histochemistry and histology of the corresponding salivary glands of species with different environment and type of feeding.

Previous investigations in several species of rodents, it was hypothesized that the differences in rodents' submandibular glands might be associated with differences in diet. The histological and histochemical changes found among the rodent species of the current study supported the hypothesis that diversity in histology and histochemistry of the sub mandibular glands originates mainly from differences of the living environments. Therefore, histochemical studies of mucopolysacharrids or enzymes of salivary glands of animals with diversified nutritional habits may refer to what the animal eats and consequently what the salivary glands secret. For instance, salivary glands of a specimen with a protein-rich diet have higher levels of protease enzymes when compared with a specimen with lower levels of protein (Flon *et al*., 1970).

However, for specimens with the same nutritional habits, namely *Allactaga ellater* and *jacullus blanfordii* of family dipodidae, the histological and histochemical differences were observed too but less extensively than others. This might issue from factors other than living environments and nutritional habits. These species live in deserts with the same environmental and feeding conditions. They all live in arid and semi-arid areas and they are gallinaceous, scarcely drink and have the maximum ability of water saving. So, there should be other factors relating to histological and histochemical differences of salivary glands in these specimens. Therefore, the differences observed between the intercalated ducts of *Allactaga ellater* and *jacullus blanfordii* that lives in similar area and same environmental and feeding conditions suggest that these differences are inherent variations rather than variations of adaptation to environment.

In this study, it was shown that salivary glands of some of specimens do not show specific adaptabilities for desert environments and they can not be distinct structurally. Therefore, it was concluded that the observed variations are most probably due to inherent variations of the specimens rather than adaptations with the environments (Junqueira *et al*., 1967).

Comparative studies of salivary glands showed that they might be related to the adaptive radiation of rodents and the relationship among different feeding habits found in rodents are possibly due to adaptive changes in a coding segment of some enzymes (Redondo and Santos, 2006).

CONCLUSION

Taken together the comparative histological characteristic features including existence of mucus and serous acini, presence of different kinds of tubules and different types of ducts as well as the histochemical characteristic features including histochemistry of different tubules and ducts in 2 different pH levels in the major and accessory sub mandibular glands in different rodents showed that the studied species proved to be different, these used comparative criteria revealed good inter-species discriminatory potential, the differences can be used very effectively in the comparative inter-species studies and these differences might be related to factors other than environment and feeding factors. The histological and histochemical characteristics of accessory and major sub mandibular glands showed that these glands are good targets structures in mammalian comparative analysis and should not be ignored by investigators. It is recommended doing comparative studies with lectin histochemistry in

accessory and major sub mandibular glands of different rodents to highlight other discriminatory characteristics in terms of function and phylogenic evaluation.

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