Histochemical and Histological Investigations on Duvernoy’s Gland in Natrix tessellata (Squamata: Colubridae) [1]

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Summary

Duvernoy’s gland, an oral gland located in temporal region, is only found in Colubrid snakes. This is the first report describing morphological and histological structure of Duvernoy's gland in Natrix tessellata (Laurenti, 1768). It is surrounded by a connective tissue layer which penetrates into the gland by forming many septa, dividing the glandular body into lobules and including the acini and the inner ducts. Duvernoy's gland is formed by seromucous acini composed of seromucous cells, and mucous acini composed of mucous cells and they are recognized by their histochemical characteristics. The gland is mainly organized in seromucous acini, mucous cells are restricted to the gland center region and to the inner secretion duct lining epithelium.

Keywords: Natrix tessellata, Colubridae, Duvernoy's gland, Seromucous acini, Mucous acini

INTRODUCTION

The oral glands, which are varies in reptiles, are located in mouth cavity and its periphery; opposite and beneath of tongue, throughout upper and lower lips, nearby of nasal cavity or at temporal region and their secretions are directed into mouth cavity. Among the reptiles, especially snakes are equipped with oral glands to immobilize the prey and lubricate the food 1-3. In snakes infralabial, supralabial glands, venom gland and Duvernoy’s or parotid glands are the most well-known oral glands 4.

The groups having real venom delivery system among contemporary reptiles are venomous snakes (Colubridae, Atractaspidae, Viperidae, Elapidae, Crotalidae) and helodermatid lizards (Heloderma suspectum and Heloderma horridum) 5,6. Mandibular gland specialized as a venom gland in helodermatid lizards extends along lower jaw. Venom is directed to mandibular teeth with many channels (Heloderma suspectum) or with a single channel (Heloderma horridum) originated from the venom gland. In venoms, all the structures of venom system (gland, muscles, fang etc.) are located along the upper jaw 7,8.

Real venom gland and Duvernoy's gland are only exist in species belonging Colubroidea superfamily 9. In fact,
both Duvernoy’s gland and real venom gland are of venom gland characteristics. Despite both types of glands are considered to be homologous, morphological and functional differences are available. Unlike Duvernoy’s gland, real venom gland consists of actual and accessory venom gland. These two parts are connected with primary channel. These parts are absent in Duvernoy’s gland and its secretion is carried with primary channel to fang groove. While real venom gland is available in species of Elapidae, Viperidae and Atractaspidae families, Duvernoy’s gland is present at some species of Colubridae family (30-40% of Colubrid snakes).

Information on function of Duvernoy’s gland is very rare. It is unusual that Duvernoy’s gland empties secretions to kill prey rapidly like a real venom gland. It is caused by the differences in excretion mechanisms of real venom gland and Duvernoy’s gland. In real venom gland, jaw adductors derived from the skeletal muscle has been included in gland capsule. Contraction of these compressor muscles are directly effective on the main venom gland. According to this hypothesis, increase of pressure as a result of the effect of compressor muscles on the gland give rise to leak of venom in pressure. In Duvernoy’s gland, jaw muscles or the ones derived from these muscles are scarcely included in gland capsule. Generally, jaw muscles pass medially without participating to gland capsule. Therefore Duvernoy’s gland secretion flows as leakage.

\textit{Natrix tessellata} is distributed all over the Turkey, Asia and Europe. Although it is abundant, there is no any histological study on Duvernoy’s gland in \textit{N.tessellata}. Aim of this study was to investigate the morphological and histological properties of Duvernoy’s gland in \textit{N. tessellata} and to prove histologically that it is a venomous species.

**MATERIAL and METHODS**

**Animals**

The protocol was approved by the animal ethics committee of Ege University, Faculty of Medicine (2009-134). Three adult individuals of \textit{Natrix tessellata} were obtained from the edges of Beyşehir Lake (between 37° 45' North latitude and 31° 36' East longitude) and were brought alive to the laboratory. Following anesthetizing with ether, Duvernoy’s gland of the snakes was dissected and processed for light microscopic studies.

**Histology and Histochemistry**

The glands were fixed in Bouin’s fixative for 24 h, dehydrated in ethanol and put to xylol for transparency, embedded in paraffin. Paraffin blocks were sectioned serially in 5 μm thickness using microtome (Baird & Tatlock). These sections were stained with Mayer’s Haematoxylin-Eosin (H&E), Periodic Acid Schiff (PAS), combined PAS and Alcian-Blue pH 2.5 and Mallory’s Trichrom. They were examined by light microscopy and then photographed with Olympus CX31-Altra 20 Soft Imaging System.

**RESULTS**

Duvernoy’s gland is thin and elongated shaped and consists of many small lobules. It measures around 8 mm long, and 2 mm wide (Fig. 2).
constituted of mucous and seromucous cells, recognized by their histochemical properties. Seromucous acini are more intensive (Fig. 3) and surround mucous acini (Fig. 4).

Periodic-Acid Schiff (PAS) histochemical method showed that mucous acini were stained dark purple but seromucous acini were stained light purple. Due to the presence of mucin, mucous cells give a strong reaction with PAS (Fig. 8).

In Haemotoxylin-Eosin staining method, mucous acini are light pink, but seromucous acini are dark purple. While mucous cells are characterized with flat basal nuclei, seromucous cells have oval basal nuclei. In addition, lumina of mucous acini is larger than seromucous acini (Fig. 5).

In Alcian Blue + Periodic Acid Schiff conjugation, Alcian blue gave weak reactions with the seromucous cells, so they stained light blue, mucous cells reacted with PAS staining pink (Fig. 10).
DISCUSSION

Although oral glands (supralabial, infralabial, Duvernoy’s gland and venom gland etc.) show diversity and are widely exist in snakes, information about morphology and function of them is very scarce. In some papers, it is cited only morphology of glands, but there is not any functional and phylogenetical peculiarities.

Both infralabial and supralabial glands have a similar function in terms of lubrication of prey during ingestion by secreting mucous. However, infralabial glands may have different functions. For example in Colubrids, a group of dipsadine snakes named “goo-eaters” have advanced infralabial glands specialized to produce mainly serous secretion in nature. In addition, Duvernoy’s gland of these snakes are not developed like in other Colubrids. So, infralabial glands in dipsadine snakes undertake similar function of Duvernoy’s gland. Consequently infralabial glands of dipsadine snakes have same histological structure in Duvernoy’s gland having both seromucous and mucous cells.

Oral glands in some dipsadine goo eaters snakes were investigated by Oliveira et al. ‘Dipsas indica’ s infralabial gland has more mucous cells when compared to Duvernoy’s gland of N. tessellata. On the other hand seromucous acini are more intensive in infralabial gland of Sibynomorphus mikanii and Atractus reticulatus like in the N. tessellata’s Duvernoy’s gland.
In view of these results, it is suggested that secretion of _D. indica_'s infralabial gland is less toxic than _N. tesellata_'s secretion. For this reason, there are some advices to increase of toxicological studies related this species in question. According to our results, mucous acini are usually limited in the center of the gland and around the internal secretion duct. A large quantity of gland is composed of seromucous acini. In _Dipsas albifrons_, mucous acini are only present in the anterior region of gland. _Duvernoy_'s gland in _N. tesellata_ shows that a large portion of the mucous cells are limited around the internal duct like in _Dipsas albifrons_ and _Sibynomorphus mikani_. In view of these data, it can be said that secretion of three species in question have toxic effects on the prey.

Periodic acid schiff (PAS) method on Duvernoy's gland of _Natrix tessellata_ indicated that mucous acini showed strong positive reaction and stained in dark purple. Same was observed in _Atractus reticulatus_. Mucous and seromucous cells are observed in same acinus in the Duvernoy's gland of _Atractus reticulatus_, _Atractus zebraurus_, _Micrurus corallinus_, and _Philodryas patagoniensis_. However, this is not the case in _Natrix tessellata_.

It is suggested that myoepithelial cells are closely related with serectory cells using transmission electron microscope in _Rhabdophis tigrinus_. In these studies, it is understood that duct epithelium comprise typical mucous secreting cells and there are low density of electron granules in the cytoplasm of these cells. This case is accordance with our results in that inner ducts comprise mucous cells in Duvernoy's gland of _Natrix tessellata_.

Recently, it is understood that venom of colubrid snakes has an toxic effect on prey's nervous system (neurotoxic). The LD<sub>50</sub> value of _Natrix tessellata_ on the mouse was determined as 25.0 (mg/kg). Venom has activities such as arginine esterase, acid phosphatase, alkaline phosphatase, protease and fosfodiesterase. Our results showed that Duvernoy's gland was composed of mainly seromucous cells and supported histologically the view related to be a venomous species of _Natrix tessellata_ which was accepted as venomous one in last years. Because of the covering of Duvernoy's gland inner duct with mucous cells and location of fang in behind of maxillary bond, we cannot say that it has a potential danger for human health. But we believe that more detailed works should be performed to reveal medicinal and pharmacological importance of Duvernoy's gland secretion.

REFERENCES