

## REVIEW ARTICLE

**Serous demilunes: Facts or fixation artefacts?**Wasim Kamate<sup>1</sup>, Nupura Vibhute<sup>2</sup>, Rajendra Baad<sup>3</sup>, Sonali Sankpal<sup>1</sup>, Rashmi Gangavati<sup>1</sup>**ABSTRACT**

In humans there are 3 bilaterally paired major salivary glands (Parotid, Submandibular and Sublingual gland) extraorally and almost 600 to 1000 minor salivary glands within the lamina propria of the mucosa of oral cavity and oropharynx. The basic functional unit of a salivary gland is the terminal secretory unit called acini (secretory end piece). In mixed salivary glands sometimes the mucous acini have bonnet or crescent shaped covering which is made of serous cells. These are called as demilunes. The discovery of demilunes is credited to Giuseppe Oronzo Giannuzzi in the year 1865. The serous demilunes are present both in major and minor salivary glands and have similar histology and ultrastructure to serous end piece cells. As the demilune cells are lying outside the acinar cells the routes by which the drainage of the demilunes is affected are not clearly understood. For more than 100 years the concept of demilunes being an actual histological entity has lasted unchallenged. However in year 1999, Yamasinha S *et al* gave a contradictory conclusion regarding the existence of demilunes as a chemical fixation artefact. However, the review of literature is more inclined towards their existence. Hence, demilunes are the basic, real structural unit of salivary glands and so rightly named 'Giannuzzi's demilunes' after its discoverer.

**Keywords:** Salivary glands; Serous demilunes; Artefacts

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**INTRODUCTION**

Salivary glands are essential for oral health. The oral cavity mucosal membrane is always kept moist by the saliva secreted by major and minor salivary glands. In humans there are 3 bilaterally paired major salivary glands (Parotid, Submandibular and Sublingual gland) extraorally and almost 600 to 1000 minor salivary glands within the lamina propria of the mucosa of oral cavity and oropharynx. They are compound, tubuloacinar, merocrine type of glands. Each of these glands has a distinct structural architecture, cellular composition and secretory products.<sup>1</sup> Depending on the histochemical nature of their secretions, salivary glands are classified as serous glands (that produce almost exclusive protein eg.: Parotid gland), mucous glands (that produce only a small amount of protein but a large amount of glycoprotein eg.: Sublingual gland and minor salivary glands) and mixed seromucous glands (that secrete both protein and glycoprotein eg: Submandibular gland).<sup>2</sup>

**Structure of terminal secretory units**

The basic functional unit of a salivary gland is the terminal secretory unit called acini (secretory end piece). This terminal secretory unit irrespective of size and location is made up of epithelial secretory cells namely serous and mucous cells. These cells along with myoepithelial cells are arranged in an acinus with a roughly spherical or tubular shape and a central lumen.<sup>3</sup>

**What are demilunes?**

Demilune literally means crescent or half moon shaped.

In mixed salivary glands, the endpiece can consist of any two of cell types- serous or mucous, in any arrangement.<sup>4</sup> In such glands, the

usual pairing of secretory cell types is that the closed terminal of the endpiece is capped by a second type of secretory cell, the demilune.<sup>5</sup>

The serous cells have been believed to locate at the most distal end of secretory end piece, surrounding the group of mucous cells forming a basophilic cap.<sup>6</sup>

In mixed salivary glands sometimes the mucous acini have bonnet or crescent shaped covering which is made of serous cells. These are called as demilunes.<sup>2</sup>

Mucous end pieces in the major salivary glands and some minor salivary glands have serous cells associated with them in the form of a demilune or crescent covering the mucous cells at the end of the tubule. These serous demilune cells are in all respects similar to the serous end piece cells present in the same gland.<sup>3</sup>

### Discovery of 'Demilunes'

In a typical mixed salivary gland, serous cells have been believed to be located at the most distal end of the secretory end-pieces, surrounding a group of mucous cells like a cap. This intriguing structure has been called Giannuzzi's demilune or crescent. The discovery of demilunes is credited to Giuseppe Oronzo Giannuzzi in the year 1865 in the introduction to the article named "The relevance of increased blood flow on the secretion of saliva" published in Leipeng at the end of his stay in Karl Ludwig's laboratory, Leipzig. By employing the histological techniques then in use (staining with carmine and intraductal injections of Prussian blue) he showed the "Halmondeformige korper" (demilunar body). Although the discovery of demilunes has occasionally been ascribed to R. Heidenhain it should be noted that this author himself recognized the Giannuzzi's priority.<sup>7</sup>

### Where are the demilunes present?

In humans demilunes are seen in the following major and minor salivary glands:

- Submandibular glands<sup>8</sup>
- Sublingual salivary glands<sup>8</sup>
- In anterior-lingual glands (glands of Blandin and Nuhn)<sup>8</sup>
- Labial and buccal minor salivary glands<sup>9</sup>

### Histology of demilunes

The mixed secretory units consist of mucous acini with serous demilunes. In H&E stained preparations, the serous cells of demilunes are pyramidal in shape, the cytoplasm shows enzyme containing zymogen granules which are strongly stained. The nuclei are rounded with dispersed chromatin and usually occupy a more central position within the cell.<sup>10</sup>

### Ultrastructure of demilunes

The serous demilunes are similar in structure to serous end piece cells.<sup>3</sup> The nucleus is central spherical, the cytoplasm contains abundant rough endoplasmic reticulum, moderate numbers of electron-dense granules<sup>11</sup> containing proteins called common salivary protein 1 (CSP-1) and neonatal submandibular gland proteins B and D (SMGB and SMGD).<sup>12</sup> The basal and lateral plasmalemma are thrown into folds and form interdigitations with adjacent cells. Intercellular canaliculi are bordered by prominent microvilli present between the demilune cells. These canaliculi are isolated from the intercellular spaces by tight junctions and desmosomes. The cells of the demilunes frequently exhibit apical extensions which pass between mucous cells to reach the acinar lumen.<sup>13</sup>

### Routes of fluid discharge

As the demilune cells are lying outside the acinar cells the routes by which the drainage of the demilunes is affected are not clearly understood.<sup>13</sup>

There seems to be two routes of discharge:

1. The demilunes cells discharge their secretions into small intercellular canaliculi that extend between the mucous cells to reach the tubule lumen.<sup>3</sup> The intercellular canaliculi interconnect in such a way that they eventually reach a demilune cell which thus has direct access to the acinar lumen.<sup>14</sup>
2. The cells of the demilunes frequently exhibit apical extensions which pass between

mucous cells to reach the acinar lumen.<sup>13</sup>

### Development of demilunes

In recent times development of demilunes has been greatly researched especially due to studies on rat salivary glands.

#### Prenatal development

The terminal buds of the developing gland acquire lumina and exhibit the first indications of secretory activity at age 18 days in utero. Through age 19 days in utero, the differentiating mucous and serous cells are typically located around a common lumen. By age 20 days in utero, serous cells begin to move peripherally to form demilunes, and the lumen is lined mainly by mucous cells. This process continues over the next day or two, and by the time of birth the basic morphological and immunocytochemical characteristics of the adult gland are established.<sup>11</sup>

Thus, all epithelial cell types characteristic of the adult gland are already present at birth, although they are still immature.<sup>12</sup>

#### Postnatal development

During the 2<sup>nd</sup> to 5<sup>th</sup> day of post natal life, immature centrally located mucous cell with serous demilunes are present. Serous demilunes contain large amount of cisternal rough endoplasmic reticulum (RER) around nucleus, small electron dense secreting granules are located close to apical region and golgi complex are less conspicuous than mucous acinar cells.<sup>15</sup>

During 5<sup>th</sup> to 20<sup>th</sup> days there is increase in number of demilune cells.<sup>15</sup> Serous demilune cell populations of sublingual gland during postnatal development grow at a rate close to that of stromal cells, but considerably higher than that of the intercalated duct and striated duct cell populations.<sup>12</sup>

Around 20<sup>th</sup> day increase in area of golgi complex is seen.<sup>15</sup>

#### Turnover and maintenance

Mature salivary glands turnover slowly. All cell types in parenchyma are found to undergo cell

division. However there is also evidence of population of stem cells which have ability of maintenance and regeneration in adult glands. Ascl3 is a transcription factor specifically localized in duct cells of salivary glands. It is found that Ascl3 expressing cells are progenitor cells of serous demilune cells of rat sublingual gland. Ascl3 can thus be used as a marker for the stem cell population.<sup>1</sup>

#### Function

Garrett JR *et al* through their study of the effects of parasympathetic and sympathetic stimulations on in situ lectin reactivity on cat submandibular gland, found that parasympathetic stimulation caused secretion of secretory glycoconjugates not only from the central acinar cells but also from the demilunar cells. In contrast, sympathetic stimulation promoted extensive depletion of secretory granules from striated ducts but only moderate secretion from the demilunar cells.<sup>17</sup>

Thus sympathetic and parasympathetic stimulation both show influence on demilunes. However, secretion of demilunar glycoproteins is more of a parasympathetic function than had been realized previously.<sup>17</sup>

#### Variation in concept of demilunes

Traditionally the concept of demilunes refers to demilunes of serous cells capping the mucous cells as seen in humans. However this relationship of acinar cells shows a variation in other species. For example, in some animals like Mastiff bat, the serous endpieces are capped by a second type of serous cells while in European hedgehog submandibular gland the serous cells have been found to be capped by mucous demilunes.<sup>17</sup>

#### The controversy: Demilunes facts or artefacts?

For more than 100 years the concept of demilunes being an actual histological entity was unchallenged.

Since its discovery in the year 1865 by Giannuzzi all the major histology textbooks have mentioned the demilunes as the real structures. However in year 1999, Yamasinha S et al gave a contradic-

tory conclusion regarding the existence of demilunes as a chemical fixation artifact.

Yamashina S et al<sup>6</sup> concluded that it was an artificial structure produced through compression by the hydrated and expanded mucous cells during immersion fixation. It was stated that due to chemical fixation the mucous cells swell to displace the serous cells towards the basal region of the acini from their original position between mucous cells rendering a structural impression in cross sections as if situated at the extreme fundus of the secretory tubule.

This study has again been interpreted by Tandler B in 2013 as flawed study. The author has argued that the demilunes are not chemical fixation artefacts but real structures.<sup>17</sup>

## CONCLUSION

Demilunes have been considered as the real differentiating structures that differentiated purely serous glands from mixed salivary glands. They form basophilic cap on the mucous cells and secrete the serous fluid. Recently controversy has challenged their existence but review of literature is more inclined towards their existence. Hence, it can be concluded safely that demilunes are the real, basic structural unit of salivary glands and so rightly named ‘Giannuzzi’s demilunes’ after its discoverer.

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