Kocatepe Veterinary Journal

Kocatepe Vet J. (2021) 14(2):255-261

DOI: 10.30607/kvj. 899909

RESEARCH ARTICLE

A Histochemical and Immunohistochemical Study on Glandula Mandibularis Mast Cells in Angora Goat

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ABSTRACT

Mast cells are particularly in association with structures, especially the skin, respiratory, and digestive systems in proximity to surfaces that interface with the external environment. This study was carry out to demonstrate the distribution and heterogeneity of mast cells in the Angora goat glandula mandibularis by using morphological, histochemical, and immunohistochemical methods. A total of seven healthy male adult Angora goats' mandibular glands were studied. Mast cells were distinctly distinguished by their metachromatic staining in preparates stained with toluidine blue. The cells were observed in various sizes and shapes, especially round, oval, and elongate-shaped. Mast cells were seen in both intralobular and interlobular interstitium in glandula mandibularis. Many mast cells were observed in the interlobular interstitium, especially around the blood vessels. The Alcian-blue/Safranin O combined staining method was used to determine mast cell heterogeneity. In glandula mandibularis, blue-colored alcian-blue (AB) (+) and pink-red colored safranin O (SO) (+) mast cell subtypes were observed. Chymase positive mast cells were usually observed one by one in both intralobular and interlobular interstitium. As a result, the mandibular gland of Angora goat which is local species was examined; the morphology, locations, heterogeneity of mast cells, and chymase expression were specified.

Key Words: Angora goat, chymase, glandula mandibularis, mast cell

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Ankara Keçisinde Glandula Mandibularis Mast Hücreleri Üzerine Histokimyasal ve İmmünohistokimyasal Bir Çalışma

ÖZ

Mast hücreleri genellikle dış çevre ile arayüz oluşturan yüzeylere yakın yapılarla, özellikle deri, solunum ve sindirim sistemleri ile ilişkilidir. Bu çalışma, Ankara keçisi glandula mandibularisinde mast hücrelerinin dağılımını ve heterojenliğini morfolojik, histokimyasal ve immünohistokimyasal yöntemlerle göstermek amacıyla yapılmıştır. Toplam yedi adet yetişkin sağlıklı erkek Ankara keçisinin glandula mandibularisi incelenmiştir. Toluidin mavisi ile boyanan kesitlerde, mast hücreleri, metakromatik boyanmaları ile belirgin bir şekilde ayırt edildi. Hücreler, özellikle yuvarlak, oval ve iğ şeklinde olmak üzere çeşitli boyut ve şekillerde gözlendi. Mast hücreleri glandula mandibularis'de hem intralobuler hem de interlobular interstisyumda görüldü. İnterlobüler interstisyumda, özellikle kan damarlarının çevresinde birçok mast hücresi gözlendi. Mast hücre heterojenliğini belirlemek için Alcian-blue / Safranin O kombine boyama yöntemi kullanıldı. Glandula mandibularis'te mavi renkli alcian-blue (AB) (+) ve pembe-kırmızı renkli safranin O (SO) (+) mast hücre alt tipleri görüldü. Kimaz pozitif mast hücreleri genellikle tek tek hem intralobüler hem de interlobüler intersitisyumda gözlendi. Sonuç olarak, yerel bir tür olan Ankara keçisinin glandula mandibularisi incelenerek; mast hücrelerinin morfolojisi, konumu, heterojenitesi ve kimaz ekspresyonu belirlendi.

Anahtar Kelimeler: Ankara keçisi, glandula mandibularis, kimaz, mast hücre

To cite this article: Ertuğrul T. A Histochemical and Immunohistochemical Study on Glandula Mandibularis Mast Cells in Angora Goat. Kocatepe Vet J. (2021) 14(2):255-261

Submission: 19.03.2021 **Accepted**: 24.05.2021 **Published Online**: 30.05.2021

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INTRODUCTION

Mast cells can be round, oval, or elongate in shape depending on their location (Chen et al. 1990), and their size varies depending on the tissue in which they are located (Ertuğrul and Kurtdede, 2017). In their cytoplasms, they have several round shape and varying in size secretion granules. Mast cells have a large, polymorphic, round, or oval-shaped nucleus in the center of the cell (Eurell and Frappier, 2006). The granules found in their cytoplasms are collected in two main groups, the substances previously synthesized and stored in the granules and substances synthesized after stimulation (Ross and Pawlina, 2016). Mast cells are particularly associated with structures close to surfaces that interface with the external environment, such as the skin, respiratory, and digestive systems (Krystel-Whittemore et al. 2016). Mast cells can function as antigen-presenting cells by processing antigens (Galli et al. 1999), and they play a key role in immune response development (Echtenacher et al. 1996), as well as innate and adaptive host immune responses to pathogens (Urb and Sheppard, 2012).

Due to granules in their cytoplasm, mast cells show metachromasia when stained with toluidine blue (Bancroft and Gamble, 2002). Mast cells were divided into two subtypes based on their physiological characteristics, staining characteristics, functional variety, mediators they contain, and responses to foreign matter release (Lin and Befus, 2002). Mucosal mast cells (MMC) and connective tissue mast cells (CTMC) are two subtypes of mast cells (Enerback, 1966). When dyed with granule-specific dyes including alcian blue and safranin O, MMC is dyed with alcian blue, while CTMC is dyed with safranin O (Chen et al. 1990). Serine proteases, biogenic amines, and chemokines are among the biologically active compounds produced and secreted by mast cells (Pejler et al. 2010). According to the distribution pattern of neutral proteases, mast cells are immunohistochemically divided into two subgroups: tryptase-positive (MC_T) and chymase-positive mast cells (MC_{TC}) (Irani and Schwarz, 1994). The acidic activation dipeptide of mast cell chymase is similar to most serine proteases found in the granule (Wolters et al. 2001). Chymase can inactivate neuropeptides and is involved in immune response (Caughey, 2007), inflammation, allergy, and angiogenesis processes (Atiakshin et al. 2019).

This study aimed to investigate the distribution and heterogeneity of mast cells in the Angora goat glandula mandibularis using histochemical and immunohistochemical methods.

MATERIAL and METHODS

Tissue samples from the mandibular glands of seven adult and healthy male Angora goats obtained from slaughterhouses in and around Ankara were used in the study. Glandula mandibularis tissue samples were fixed in a 10% formaldehyde solution for 24 hours. The tissues were held for 1 hour in each of 70 percent, 80 percent, and 96 percent alcohol after being stored in a running water bath for 24 hours to remove the formalin. This was followed by three 1 hour applications of absolute alcohol and xylol were done. The tissue samples were then blocked in paraffin.

Histochemistry

From the prepared blocks, 5 μm thick sections were taken and stained with toluidine blue (0.5%, pH 0.5) prepared in McIlvaine's citric acid disodium phosphate buffer to show the mast cells for 10 minutes (Enerback, 1966). Also, sections taken from blocks were stained in 0.2 M acetate buffer alcian blue (0.5%, pH 0.2) /safranin O (0.25%, pH 1.42) combined dyes to determine the subtypes of mast cells and their distributions in tissues (Bancroft and Cook, 1984).

Following the staining alcian blue/safranin O combined method, the number of AB (+) and SO (+) mast cells in $1mm^2$ was evaluated semi-quantitatively as (+), (++), and (+++).

Immunohistochemistry

Anti-rabbit polyclonal chymase (1/200 dilution, Biorbyt, orb11030) primary antibody was used to stain 5 m thick mandibular gland sections taken from paraffin blocks using the Streptavidin biotin complex method (True, 1990). Histostain Plus (Zymed kit: 85-6743) kit was used as the secondary antibody. After deparaffinization, sections were heated in a microwave oven of 700 watts within citrate buffer (pH 6) solution for antigen retrieval. In order to block endogenous peroxidase activity, the tissues were incubated in a 3% hydrogen peroxide solution. Following washing with phosphate buffer solution (PBS), serum in the kit was instilled to prevent nonspecific protein binding in sections. Primary antibody was applied to sections and they were stored at +4 ^{0}C overnight. Only PBS solution was instilled on negative control group tissues. Following the washing procedure, biotinylated secondary antibody was instilled into sections and incubated at streptavidin-horseradish peroxidase complex after washing. In the last stage, 3, 3'-diaminobenzidine (DAB) was used as chromogen, and counterstaining was performed with hematoxylin.

RESULTS

Histochemical Findings

Toluidine Blue Staining

Mast cells were easily distinguished in light-microscopic examinations of tissue cross-sections taken from the glandula mandibularis of the Angora goat due to metachromasia. The cells were observed

in a variety of sizes and shapes, particularly round, oval, and elongate-shaped. Mast cell nuclei were found to be centrally and eccentrically located and were covered by granules in the most of cells. Mast cell granules could not be identified one by one in the majority of the sections examined because the cells' cytoplasm was homogeneously stained. Mast cells were found in the glandula mandibularis' intralobular and interlobular interstitium. Mast cells were seen more in the interlobular interstitium. Mast cells were found in dense clusters around salivary secretory ducts. Many mast cells were observed in the connective tissue between the lobes, particularly around the blood vessels (Fig. 1).

AB/SO Combine Staining

In the glandula mandibularis, blue color AB (+) and pink-red color SO (+) mast cells were identified using the AB/SO staining method to determine mast cell heterogeneity. AB (+) and SO (+) were found to be round and elongate-shaped. AB (+) and SO (+) cells were observed in the glandula mandibularis, close to the salivary ducts, in both the intralobular and interlobular interstitium (Fig. 2). It was seen that mast cells stained SO (+) in the glandula mandibularis were more than AB (+) stained mast cells (Table 1).

Immunohistochemical Findings

Chymase-Positive Mast Cell

Chymase-positive mast cells with oval and round shapes were found in the glandula mandibularis. MC_{TC}s were observed one by one in both intralobular and interlobular interstitial spaces. MC_{TC}s in the mandibular gland were seen around the vessels and in the connective tissue septa covering the salivary secretory ducts. Also, MC_{TC}s were observed to be mostly located near the glands (Fig. 3).

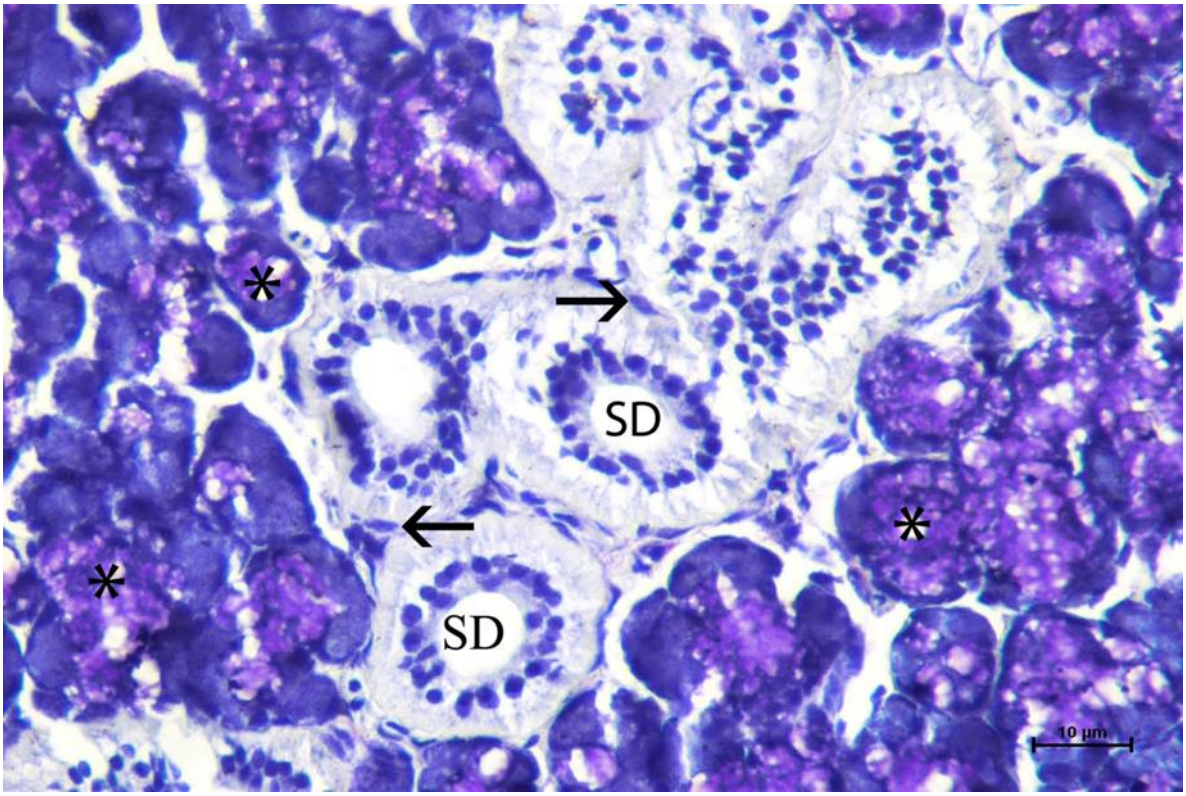

Figure 1: Toluidine blue staining; arrow: metachromatic mast cells, glandula (asterisk), secretory ducts (SD), original magnification X40; range bar, 10 µm.

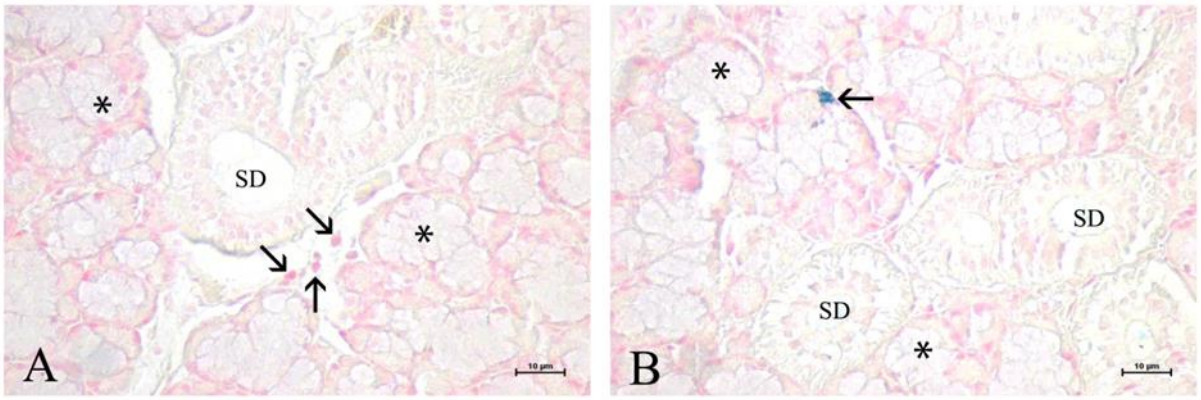

Figure 2: Alcian blue/safranin O combined staining method; A, arrow: SO (+) mast cell, glandula (asterisk), secretory ducts (SD), B, arrow: AB (+) mast cell, glandula (asterisk), secretory ducts (SD), original magnification X40; range bar, 10 μm.

Table 1. The density of AB (+) and SO (+) mast cells in glandula mandibularis (alcian blue/safranin O combined method, semi-quantitatively evaluating (+), (++) and (+++)).

Mast cell	Density
Alcian blue (+)	(+)
Safranin O (+)	(++)

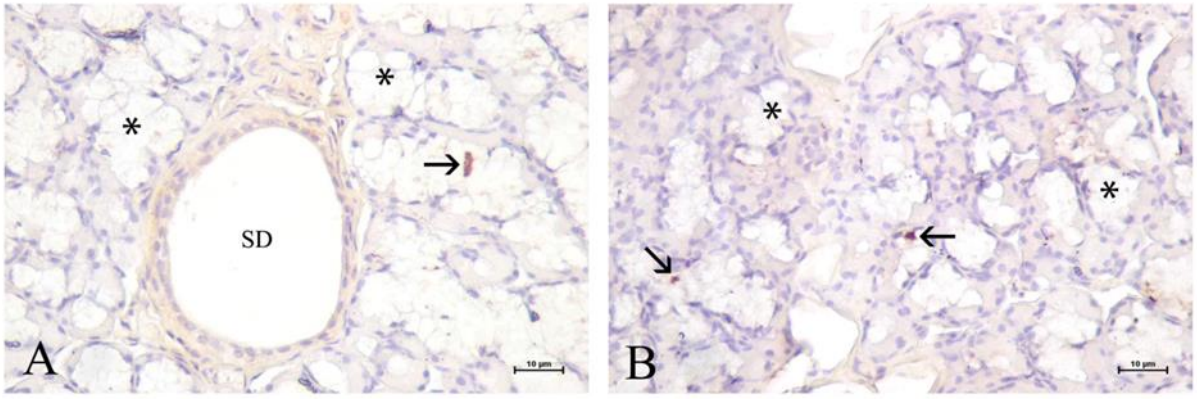

Figure 3: Chymase immunostaining A, arrow: oval-shaped MC_{TC}, glandula (asterisk), secretory ducts (SD), B, arrow: round-shaped MC_{TC}, glandula (asterisk), original magnification X40; range bar, 10 μm.

DISCUSSION

A large number of foreign antigenic substances come into contact with the digestive system. Mast cells are abundant in systems exposed to the outside world, and they are among the first cell groups to respond to pathogens and foreign body entry (Ertugrul et al. 2018). According to researches, mast cells in the salivary glands play an essential role in salivary antigen detection, and they collaborate closely with other antigen-presenting cells, including dendritic cells (David et al. 2010).

When stained with toluidine blue, mast cells exhibit metacromasia due to granules in their cytoplasm (Bancroft and Gamble, 2002), and they can be round, oval, or spindle-shaped depending on where they are in the tissues (Chen et al. 1990). In studies of goats' lower respiratory tract (Kurtdede et al. 2000), female reproductive organs (Karaca et al. 2009), and urinary system (Ertuğrul and Kurtdede, 2017), mast cells were observed metachromatically stained with toluidine blue. According to studies conducted in the digestive system (Uslu and Yörük, 2008), tongue (Ertugrul et al. 2018), and goat respiratory (Kurtdede et al. 2000) and urinary systems (Ertuğrul and Kurtdede, 2017), mast cells were round, oval, or spindle-shaped, and the nuclei were located centrally or eccentrically. Our findings are consistent with previous research in terms of staining characteristics and morphological properties of mast cells. Mast cells have been observed in the stroma of the submandibular gland, particularly around the vessel, as well as in the connective tissue septa surrounding the gland tissue (Henriksson et al. 1994). According to a study, it was reported that there are a large number of mast cells in the interlobular interstitium of the glandula parotis in rats, especially around the vessels and secretory ducts of the gland (Altunlu et al. 2010). The findings of the researchers on the location of mast cells in the glandula mandibularis are similar to ours.

Mast cell heterogeneity is histochemically classified based on their origins, glycosaminoglycan types, intragranular serine proteinase type (Atkins et al. 1985), histochemical differences, and functional criteria into two subgroups as MMC and CTMC (Hunt et al. 1991). As alcian-blue safranin O is stained sequentially, MMC gives a positive reaction with alcian blue, while CTMC gives a positive reaction with safranin O (Chen et al. 1990). AB (+) mast cells were reported in a study investigating mast cell heterogeneity in the goat urinary system (Ertuğrul and Kurtdede, 2017). Studies have been carried out to determine mast cell heterogeneity in the digestive system (Karaca and Yörük, 2004), proventriculus (Aksoy and Çınar, 2008), and tongue (Fletcher and Triantafyllou, 2007). Mast cells were stained in two subtypes, blue-colored AB (+) and pink-red colored SO (+), in tissues using the AB/SO combined staining method, according to these studies. In glandula mandibularis, we determined two subtypes of mast cells with SO (+) and AB (+) staining. The heterogeneity of mast cells in Angora goats in our study is similar to that found in the previous studies. Also, based on our findings, we observed that CTMCs outnumbered MMCs in the glandula mandibularis.

Mast cell chymase stimulates homeostasis by limiting the toxicity of toxic endogenous peptides (Maurer et al. 2004). Chymase can regulate fibrous tissue accumulation in scar formation (Matsumoto et al. 2003) and also may modulate extracellular matrix remodeling (Fang et al. 1997). It has also been determined to play a role in inflammation, allergy, and angiogenesis mechanisms (Atiakshin et al. 2019). When tissue damage occurs, chymase levels increase, attracting neutrophils to the wound site (Wulff and Wilgus, 2013). In a study of the lingual salivary gland, MC_{TC}s were seen around the salivary gland ducts (Bertoldo et al. 2019). Furthermore, previous research has shown that MC_{TC} is the most common mast cell population in the oral mucosa and dental pulp (Walsh et al. 1995). MC_{TC} cells in the oral cavity have been shown to have a subepithelial localization in the oral mucosa (Yadav et al.2014). Mast cells found in the connective tissue between salivary secretion ducts were found to be distributed one by one in our study. One of the first areas pathogens and other foreign agents come into contact with the organism is in the oral cavity. The existence of MC_{TC}s in the salivary gland indicates that these cells may play a role in the body's defense system. It is concluded that the histological findings obtained from the MC_{TC}s are an important structural element in the oral cavity defense mechanism.

CONCLUSION

As a result, the mandibular gland of an Angora goat, a local species, was investigated; the morphology, locations, heterogeneity of mast cells, and chymase were all attempted to be specified. This study's results appeared to be in line with previous studies on mast cell properties in a variety of species. Because there were not many studies about mast cells of glandula mandibularis of Angora goat, it is thought that the findings of this study will contribute to the literature. Based on the histological findings, it can be concluded that mast cells play an essential role in the digestive system's defense mechanisms and allergic inflammatory reactions.

Ethics Committee Information: This study is not subject to HADYEK's permission in accordance with Article 8 (k) of the "Regulation on Working Procedures and Principles of Animal Experiments

Ethics Committees". In addition, the authors declared that they comply with the Research and Publication Ethics.

Conflict of Interest: The authors declared that there is no conflict of interest.

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