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Mahmut Sozmen, Peter J. Brown, Peter J. Cripps. Quantitation of histochemical staining of salivary gland mucin using image analysis in cats and dogs. *Veterinary Research*, 1999, 30 (1), pp.99-108. <hal-00902560>

HAL Id: hal-00902560

<https://hal.science/hal-00902560v1>

Submitted on 11 May 2020

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Quantitation of histochemical staining of salivary gland mucin using image analysis in cats and dogs

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(Received 1 October 1997; accepted 17 September 1998)

Abstract – Two different, computer-based, image analysis methods were employed to complement subjective assessment of patterns of mucin staining (by periodic acid Schiff/alcian blue, aldehyde fuchsin/alcian blue and potassium hydroxide-alcian blue/phenylhydrazine hydrochloride) in digitised images of sections of major and minor salivary glands from cats and dogs. Image analysis based on red, green and blue (RGB) staining was not suitable for quantitation of histochemical staining of mucin in salivary glands. Image analysis based on hue, saturation and lightness (HSL) allowed quantitative assessment of staining by different stain components and of mixed staining, and enabled comparison of staining of different glands in dogs and cats. Quantitative analysis based on HSL allowed differentiation of differences in staining patterns of major and minor cat and dog salivary glands, and between the species; such differences would have been impossible to distinguish on subjective grounds alone. Quantitative assessment of normal salivary gland histochemistry allows comparison with staining patterns in disease. © Inra/Elsevier, Paris.

morphometry / image analysis / cat / dog / salivary gland

Résumé – **Quantification par analyse d'image de la coloration histochimique des mucines des glandes salivaires de chien et de chat.** Deux méthodes différentes d'analyse d'image sur ordinateur ont été utilisées pour compléter l'appréciation subjective des modes de coloration des mucines (par acide périodique de Schiff/bleu alcian, fuchsine aldéhyde/bleu alcian et hydroxyde de potassium-bleu alcian/hydrochlorure de phénylhydrazine) à partir d'images digitalisées de coupes des glandes salivaires majeures et mineures provenant de chiens et de chats. L'analyse d'image fondée sur la coloration bleu, vert et rouge (*red, green and blue*: RGB) n'a pas permis de quantifier la coloration histochimique des glandes salivaires. L'analyse d'image fondée sur la teinte, la saturation et la brillance (*hue, saturation and lightness*: HSL) a permis d'une part de quantifier le marquage par les différents composants des colorations et par leur mélange et d'autre part de comparer le marquage des diverses

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glandes chez les chiens et les chats. L'analyse d'image fondée sur la méthode HSL a permis la distinction de différents modes de coloration des glandes salivaires majeures et mineures chez le chien et le chat et entre espèces ; de telles distinctions n'auraient pas pu être obtenues à partir des seules données subjectives. L'appréciation quantitative de l'histochimie de glandes salivaires normales permet la comparaison avec les colorations obtenues lors de maladies. © Inra/Elsevier, Paris.

morphométrie / analyse d'image / chat / chien / glande salivaire

1. INTRODUCTION

Combinations of histochemical stains allow simultaneous demonstration of different types of mucins. Examples of these methods are periodic acid Schiff/alcian blue (PAS/AB); high iron diamine/alcian blue (HID/AB): PAS stains neutral mucins; alcianophilia is attributable to the presence of carboxyl groups; HID selectively stains sulphated mucosubstances. A combined aldehyde fuchsin/alcian blue (AF/AB) technique provides an alternative to the high iron diamine-alcian blue [17].

Previous studies of salivary gland histochemistry of cats and dogs have been qualitative and subjective [10, 12, 20]. Such an approach is valuable and plays a major role in investigation of normal tissues and in disease diagnosis. In other situations, estimation of size and tissue distribution of particular cell components is important and morphometrical analysis has increased in recent years. Most morphometrical analyses have utilised the stereological point-counting technique for assessing proportional volumes of salivary constituents; in people, they have included an assessment of the replacement of salivary gland parenchyma by fibrous and fatty tissue as an effect of ageing [3, 14] or to detect morphometrical changes to justify a diagnosis of Sjogren's syndrome [1]. Morphometrical studies in animal salivary glands are few [4, 13]. No quantitative data are available to show the proportional amounts of different mucins secreted by cat and dog salivary glands.

This study was undertaken to determine the distribution of different mucins and to

measure the relative percentages of different classes of mucosubstances in normal major and minor salivary glands in cats and dogs. The histochemical methods used allowed mucins to be recognised within three major categories within the classification of Spicer et al. [19]: neutral mucins, sulphomucins and sialomucins. Two different analyses were available within the image analysis package employed; these were based on threshold extraction imagery of the three primary colours – red, green and blue (RGB), and threshold imagery based on hue, saturation and lightness (HSL).

2. MATERIALS AND METHODS

2.1. Animals and tissues

Six samples of normal tissue from the palatine, parotid, sublingual, submandibular, tongue and zygomatic glands were removed during the course of post-mortem examination from a total of 13 dogs [16] and ten cats; in the cat samples stained with AB/PAS it was possible to consider the serous von Ebner's glands separately from the more extensive mixed glands. In all cases, the cause of death or euthanasia was not related to salivary gland disease and the glands were macroscopically normal. Details of the animals are given in *table 1*.

The tissues were fixed in 10 % neutral buffered formol saline for 1–2 days at room temperature before being routinely processed and embedded in polywax at 60 °C. Sections were cut at 5 µm thickness and mounted on slides. Representative sections were stained with haematoxylin and eosin to confirm their normal structure.

Table I. Age, sex, breed and disease background of the 13 dogs and ten cats studied.

| Animal | Age (year) | Sex | Breed ^a | Diagnosis confirmed at PM | Glands examined ^b | | | | | | | |
|--------|---------------|------|--------------------|--------------------------------|------------------------------|----|----|---|----|---|--|--|
| | | | | | SL | PR | SM | Z | PL | T | | |
| Dog | | | | | | | | | | | | |
| 1 | 6 | F(N) | JRT | perivascular cuffing | + | + | + | + | + | - | | |
| 2 | 3 | F | WSS | cirrhosis | + | - | + | + | + | - | | |
| 3 | 7 | F(N) | CKCS | retropharyngeal tumour | + | + | + | + | + | - | | |
| 4 | 7 | M | cross | disc prolapse | + | + | + | + | + | - | | |
| 5 | 8 | M(N) | FCR | lymphoma | + | + | + | + | - | - | | |
| 6 | 3 | F(N) | Whippet | inflammation with abscess | + | + | + | + | + | - | | |
| 7 | 11 | M | Labrador | haemangiosarcoma | - | + | - | - | - | - | | |
| 8 | 8.9 | F | Shetland | inhalational pneumonia | - | - | - | - | + | + | | |
| 9 | 8 | (N) | ESS | anaemia | - | - | - | - | - | + | | |
| 10 | 5 | M | cross | renal infarction | - | - | - | - | - | + | | |
| 11 | 4 | F(N) | cross | pyloric ulceration | - | - | - | - | - | + | | |
| 12 | 11 | F(N) | Keeshund | pneumonia | - | - | - | - | - | + | | |
| 13 | 5 | F | Great Dane | chondrosarcoma | - | - | - | - | - | + | | |
| Cat | | | | | | | | | | | | |
| 1 | 0.6 | M | Birman | nephropathy | + | + | + | + | - | - | | |
| 2 | 11 | M(N) | DSH | meningoencephalitis | + | + | + | + | + | + | | |
| 3 | 3 | F(N) | DSH | cardiomyopathy | - | + | - | + | - | + | | |
| 4 | 7 | F(N) | DSH | adenocarcinoma | - | - | + | + | - | + | | |
| 5 | 9 | F | DSH | ceruminous adenocarcinoma | + | + | + | + | + | + | | |
| 6 | 4.5 | F | Chinchilla | pneumonia | + | + | + | + | - | + | | |
| 7 | 6 | F(N) | Siamese | chronic interstitial nephritis | - | + | + | - | + | + | | |
| 8 | 7 | M(N) | DSH | pituitary adenoma | + | - | - | - | + | - | | |
| 9 | 11 | F(N) | DSH | multicentric lymphoma | + | - | - | - | + | - | | |
| 10 | 4 | F | Siamese | chronic interstitial nephritis | - | - | - | - | + | - | | |

^a JRT: Jack Russel Terrier; WSS: Welsh Springer Spaniel; CKCS: Cavalier King Charles Spaniel; FCR: Flat Coat Retriever; ESS: English Springer Spaniel; DSH: Domestic Short Hair.

^b SL: sublingual; PR: parotid; SM: submandibular; Z: zygomatic; PL: palatine; T: tongue; N = neutered.

2.2. Mucosubstance histochemical procedures

De-waxed sections were stained, at room temperature, with:

1) alcian blue/periodic acid Schiff (AB/PAS) [9]:

1 % alcian blue (pH 2.5) solution for 5 min;
1 % periodic acid for 5 min; rinsed and exposed to Schiff's reagent for 15 min; Mayer's haematoxylin counterstain;

2) aldehyde fuchsin/alcian blue (AF/AB) [18]:
aldehyde fuchsin solution for 20 min; alcian blue (1 %) solution (pH 2.5) for 5 min;

3) potassium hydroxide/AB/periodic acid phenylhydrazine Schiff (KOH/AB/PAPS) [11]:

0.5 % KOH in 70 % (v/v) aqueous ethanol for 15 min; alcian blue (1 %) in 0.1 M HCL, pH 1.0 for 30 min; 1 % (w/v) periodic acid for 2 h; 0.5 % aqueous phenylhydrazine hydrochloride for 2 h; basic fuchsin Schiff for 4 h.

2.3. Morphometrical analysis

Digitised images of stained sections were captured using a Videovue capture card (Video Associates Labs Inc.; Austin, Texas, USA) installed in a 486, DX266 computer linked to a microscope (Nikon microphot-fxa.) with a one chip colour video camera. Images were obtained as 10 × 40, full colour (24 bit).tif files.

The captured images were investigated on a 6100/60 Power Macintosh computer using a semiautomatic image analysis program (Optilab 2.5; ME Electronics, Reading, UK). Images were analysed following thresholding in terms of RGB and HSL.

2.3.1. Image analysis based on RGB

RGB threshold analysis was performed using an automatic function of the analysis software package as well as by manipulation of individual red (R), green (G) and blue (B) values.

2.3.2. Image analysis based on HSL

Images were subjected to threshold analysis in terms of H, S and L. H values were chosen which distinguished staining with each individual stain, and combined staining, which correlated with that observed on subjective assessment. The same values were used for all sections stained by the same stain combination and are presented in *table II*.

Two different areas (each representing a tissue section area of about 0.640 mm²) were measured from each section (gland/stain combination). Areas were selected to include secretory tissue and associated ducts but to exclude larger blood vessels and interlobular/interlobar connective tissue. Results were expressed as median percentage stained area.

Data were analysed using Minitab (version 9.2; 1993) and differences were tested by using non-parametric (Kruskal Wallis) statistical analyses, group means were compared using Mann-Whitney rank sum test. A null hypothesis of no difference was used and a critical probability $P = 0.05$ was assumed.

3. RESULTS

3.1. Subjective assessment of mucin histochemical staining characteristics

Three general types of epithelial mucosubstances were identified by the histochemical techniques employed; in broad terms, they were recognised as belonging to three major categories: neutral mucins, sulphomucins and sialomucins.

AB/PAS: neutral mucins were identified by their positive (red) PAS reaction. Acidic

Table II. Hue, saturation and lightness values employed in analysis of salivary gland mucin histochemical staining.

| Staining method | Stain result | H (hue) | S (saturation) | L (lightness) |
|-------------------|--------------|---------|----------------|---------------|
| AB/PAS stain | AB | 120–169 | 0–255 | 0–255 |
| | AB/PAS | 170–200 | 0–255 | 0–255 |
| | PAS | 201–219 | 0–255 | 0–255 |
| AF/AB stain | AF | 201–219 | 0–255 | 0–255 |
| | AF/AB | 150–200 | 0–255 | 0–255 |
| | AB | 100–149 | 0–255 | 0–255 |
| KOH/AB/PAPS stain | KOH/AB | 120–155 | 0–255 | 0–255 |
| | AB/PAPS | 156–200 | 0–255 | 0–255 |
| | PAPS | 201–230 | 0–255 | 0–255 |

mucins, stained by alcian blue alone, appeared sky blue. Those mucins stained by both stains varied in colour from reddish purple to royal blue, depending on the degree of alcianophilia.

AF/AB: sulphomucins were stained various shades of purple; acidic mucins appeared blue. Mixed staining exhibited various shades of purple-bluish, depending on the proportions of carboxylated and sulphated mucins.

KOH/AB/PAPS: the results were similar to those given by the AF/AB method except that sulphomucins were stained aquamarine instead of reddish or bluish purple.

Differences among histochemically demonstrable salivary mucins were both qualitative and quantitative. Only minor differences in staining characteristics were noted in the same glands obtained from different subjects. Subjective assessment of the distributions and relative proportions of different types of mucin in the different salivary glands are summarised in *table III*.

3.2. Morphometrical analysis

3.2.1. Image analysis based on RGB

No combination of RGB thresholds was found which selectively identified staining of different components or mixed staining.

3.2.2. Image analysis based on HSL

It was possible to select H values which distinguished staining with each individual stain, and combined staining, which correlated with that observed subjectively (*table III*). There were marked differences between the proportions of different glands stained by the various stain components, and combinations, in cats and dogs. The results (median values \pm SD) are presented in *table IV*. Statistical analyses of the results are presented in *tables V–VII*.

Differences in staining pattern between the various cat and dog salivary glands are shown in *tables V–VII*. There were few differences between cat and dog glands with AB/PAS staining (*table V*); there were more differences between cat and dog glands stained with AF/AB (*table VI*) and KOH/AB/PAPS (*table VII*).

4. DISCUSSION

In the present study, three general types of epithelial mucosubstances were identified; in broad terms, they were recognised as neutral mucins, acidic sulphated mucins and acidic sialylated mucins according to the classification of Leppi and Spicer [6] and Spicer et al. [19], who categorised mucins into four histochemical groups. Type I mucins are characterised by PAS-positivity and negligible staining with cationic dyes. Type II mucins are carboxylated and also react with PAS but, in addition, bind alcian blue; they fail to react with the HID reagents. Types III and IV mucins are PAS-positive, alcianophilic and stain positively with HID reagents, with differences in the intensity of cationic dye binding.

The significance of the different types of secretions in different salivary epithelial cells remains unknown. Menghi et al. [8] observed that changes of diet do not affect the type of secretion by postnatal rabbits while sialic acid content increased with growth. Differences in chemical components (amylase) from rat parotid saliva have been shown to vary in response to changes in diet and the relationship of diet to mastication [5]. In the present investigation, some differences in staining characteristics were noted in the same glands obtained from different subjects; variations in staining may be indicative of different stages in secretory cycles [2].

The distribution of different staining patterns in cats and dogs reported in the present study are in general agreement with results of other workers [10, 12, 15, 20]. Some

minor differences were noted on the basis of morphometrical analysis. For example, cat submandibular and sublingual gland are both mixed (serous and mucous) glands; Tachi [20] indicated that mucous cells predominate in the submandibular gland while serous cells are more numerous in the sublingual glands. The quantitative approach

employed in the present study indicated that sulphated mucous secretion predominated in both submandibular and sublingual glands (69.3 and 22.3 % KOH/AB-positive staining with KOH/AB/PAPS, respectively). The difference between those figures and the findings of Tachi [20] may be that what, on simple microscopic observation,

Table III. Summarised subjective assessment of staining characteristics of acinar and demilune cells in different dog and cat salivary glands.

| Gland* | Tissue component | Staining characteristics | | |
|--------|------------------|--------------------------|-------------------------------|-------------------------------------|
| | | AB/PAS acid/neutral | AF/AB sulphated/sialylated | KOH/AB/PAPS sulphated/sialylated |
| Dog | | | | |
| PL | acinar cells | neutral > acid | sulphated > sialylated | sulphated |
| | demilune cells | neutral | - | - |
| PR | acinar cells | neutral > acid | sialylated | sialylated |
| | demilune cells | na | na | na |
| SL | acinar cells | neutral > acid | sialylated > sulphated | sialylated > sulphated |
| | demilune cells | neutral | - | sialylated |
| SM | acinar cells | neutral > acid | sulphated > sialylated | sulphated |
| | demilune cells | acid | sialylated; sulphated | sialylated; sulphated |
| T | acinar cells | acid > neutral | sulphated > sialylated | sulphated > sialylated |
| | demilune cells | neutral | - | - |
| Z | acinar cells | neutral > acid | sulphated > sialylated | sulphated |
| | demilune cells | neutral | - | - |
| Cat | | | | |
| PL | acinar cells | neutral > acid | sulphated | sulphated |
| | demilune cells | neutral | - | - |
| PR | acinar cells | neutral > acid | sialylated > sulphated | sialylated > sulphated |
| | demilune cells | na | na | na |
| SL | acinar cells | neutral > acid | sulphated > sialylated | sulphated > sialylated |
| | demilune cells | PAS | - | - |
| SM | acinar cells | acid > neutral | sulphated | sulphated |
| | demilune cells | acid | sulphated; sialylated | sulphated; sialylated |
| T | acinar cells | acid > neutral | sulphated > sialylated | sulphated > sialylated |
| | demilune cells | neutral | - | - |
| TV | acinar cells | neutral | - | - |
| | demilune cells | na | - | - |
| Z | acinar cells | neutral > acid | sulphated | sulphated |
| | demilune cells | neutral | - | - |

na: not applicable (cell type absent from the gland).

-: staining was negative with the method applied.

* PL: palatine; PR: parotid; SL: sublingual; SM: submandibular; T: tongue; TV: tongue (von Ebner's); Z: zygomatic gland.

Table IV. Percentage (median values) of mucin classes of different salivary glands stained with AB/PAS, AF/AB and KOH/AB/PAPS.

| Gland* | Stain combination/component | | | | | | | | |
|------------|-----------------------------|-----------|-----------|-----------|-----------|-----------|-------------|-----------|-----------|
| | AB/PAS | | | AF/AB | | | KOH/AB/PAPS | | |
| | AB | mixed | PAS | AF | mixed | AB | KOH/AB | mixed | PAPS |
| Dog | | | | | | | | | |
| PL | 0 ± 3 | 65.8 ± 10 | 19.5 ± 8 | 12.3 ± 22 | 49 ± 13 | 0.8 ± 6 | 64.8 ± 22 | 0.5 ± 16 | 0 ± 0.2 |
| PR | 0 ± 0 | 7 ± 17 | 41.8 ± 17 | 0 ± 0 | 0 ± 0 | 27.8 ± 10 | 0 ± 0.6 | 0 ± 2 | 27.8 ± 18 |
| SL | 0 ± 0 | 29.5 ± 14 | 27.8 ± 10 | 0.3 ± 2 | 1.8 ± 9 | 20.5 ± 4 | 0.5 ± 5 | 25 ± 16 | 4.5 ± 3 |
| SM | 0 ± 4 | 74.5 ± 6 | 2.5 ± 2 | 2.75 ± 3 | 60 ± 13 | 2 ± 8 | 66.8 ± 18 | 11.5 ± 21 | 0 ± 1 |
| T | 28.2 ± 41 | 31.7 ± 31 | 3.8 ± 6 | 31.3 ± 23 | 18.8 ± 13 | 0 ± 9 | 69 ± 11 | 0.5 ± 5 | 0 ± 0 |
| Z | 0 ± 0 | 47 ± 17 | 34.8 ± 19 | 13.8 ± 10 | 66.8 ± 20 | 0 ± 1 | 83.5 ± 11 | 0 ± 0 | 0 ± 0 |
| Cat | | | | | | | | | |
| PL | 0 ± 4 | 22 ± 21 | 48 ± 24 | 44 ± 27 | 32.5 ± 20 | 1 ± 21 | 71.5 ± 17 | 1 ± 12 | 0 ± 0 |
| PR | 0 ± 0 | 29 ± 20 | 57.3 ± 18 | 0 ± 2 | 21.8 ± 22 | 38.8 ± 14 | 0.3 ± 17 | 33.5 ± 22 | 1.5 ± 3 |
| SL | 0 ± 0 | 7.3 ± 16 | 26 ± 14 | 5.8 ± 8 | 10.8 ± 19 | 0.5 ± 1 | 22.3 ± 13 | 0 ± 10 | 0 ± 0.4 |
| SM | 43.8 ± 15 | 33.3 ± 20 | 0.8 ± 1 | 19.3 ± 15 | 27 ± 9 | 0 ± 3 | 69.3 ± 12 | 0 ± 0 | 0 ± 0 |
| T | 6.5 ± 20 | 53 ± 16 | 5.3 ± 3 | 17.3 ± 20 | 32.3 ± 21 | 0.5 ± 14 | 28.8 ± 20 | 0.8 ± 1 | 5 ± 3 |
| TV | 0 ± 0 | 0 ± 0 | 33.3 ± 20 | NS | NS | NS | NS | NS | NS |
| Z | 0 ± 0.6 | 19.5 ± 21 | 55 ± 15 | 55.3 ± 13 | 3 ± 8 | 0 ± 0 | 73 ± 25 | 0 ± 17 | 0 ± 0.2 |

NS: staining was negative with the method applied.

NSS: not sufficient specimen.

* PL: palatine; PR: parotid; SL: sublingual; SM: submandibular; T: tongue; TV: tongue (von Ebner's), Z: zygomatic gland.

appeared to be solely sialomucin was, on analysis, mixed with sulphated mucin. In a similar way, Reifel and Travill [12] found that all dog parotid gland acini were alcianophilic (with AB/PAS) indicating the presence of mainly acidic carbohydrates. In this investigation, no purely alcianophilic staining was detected and PAS-positive staining dominated (41.8 %). This discrepancy might reflect small amounts of PAS-positivity in the cytoplasm resulting in alcian blue-positive staining being recognised as mixed staining. The quantity of PAS positive secretory granules is affected by the phase of the secretory cycle; the secretory granules are concentrated during a phase of maturation, after enzyme synthesis and before discharge of secretion [2].

The mucous acinar cells present in cat and dog minor salivary glands are a mixed population mainly producing sulphated mucosubstances, which are histochemically similar to comparable cells in the sublingual and submandibular glands. On the histochemical basis of the present study, the acinar cells of all minor salivary glands are best categorised as mucous, since the cells contain neutral and acidic mucopolysaccharides with mainly sulphomucin and sialomucin.

In many glands in the present study, the staining patterns included staining with each component stain alone, as well as combined staining; morphometric analysis allowed further discrimination of the mucin staining patterns in different glands. Such a

Table V. Differences (*P* values) of staining characteristics (AB positive; mixed staining and PAS positive) of different salivary glands stained with AB/PAS.

| Gland* | SM | PR | SL | Z | PL | T | TV |
|-----------------------|-------|-------|-------|-------|-------|-------|-------|
| AB staining | | | | | | | |
| SM | 0.004 | 0.002 | 0.002 | 0.004 | 0.004 | 0.045 | NA |
| PR | NS | NS | NS | NS | NS | 0.008 | NA |
| SL | NS | NS | NS | NS | NS | 0.008 | NA |
| Z | NS | NS | NS | NS | NS | 0.038 | NA |
| PL | NS | NS | NS | NS | NS | NS | NA |
| T | 0.025 | 0.008 | 0.008 | 0.008 | 0.025 | NS | NA |
| Mixed staining | | | | | | | |
| SM | 0.005 | NS | 0.045 | NS | NS | NS | 0.005 |
| PR | 0.005 | NS | NS | NS | NS | 0.03 | NS |
| SL | 0.005 | NS | NS | NS | NS | 0.008 | NS |
| Z | 0.005 | 0.045 | NS | NS | NS | 0.045 | NS |
| PL | 0.03 | 0.005 | 0.005 | 0.045 | 0.013 | NS | NS |
| T | 0.005 | NS | NS | NS | NS | NS | 0.005 |
| PAS staining | | | | | | | |
| SM | NS | 0.005 | 0.05 | 0.05 | 0.005 | NS | NA |
| PR | 0.005 | NS | 0.013 | NS | NS | 0.005 | NA |
| SL | 0.005 | 0.029 | NS | 0.02 | NS | 0.05 | NA |
| Z | 0.005 | NS | NS | NS | NS | 0.005 | NA |
| PL | 0.005 | 0.008 | NS | NS | NS | 0.025 | NA |
| T | NS | 0.005 | 0.02 | 0.008 | 0.02 | NS | NA |

Between glands in dogs below the shaded boxes; between glands in cats above the shaded boxes; between cats and dogs for each gland in the shaded boxes.

* SM: submandibular; PR: parotid; SL: sublingual; Z: zygomatic; PL: palatine; T: tongue; TV: Tongue (von Ebner's).

NS: not significant; NA: not applicable.

Table VI. Differences (*P* values) of staining characteristics (AF positive; mixed staining and AB positive) of different salivary glands stained with AF/AB.

| Gland* | SM | PR | SL | Z | PL | T |
|-----------------------|--------|-------|-------|-------|-------|-------|
| AF staining | | | | | | |
| SM | 0.0064 | 0.004 | NS | 0.005 | NS | NS |
| PR | 0.002 | NS | 0.022 | 0.004 | 0.009 | 0.007 |
| SL | 0.036 | NS | 0.029 | 0.005 | NS | NS |
| Z | 0.013 | 0.002 | 0.005 | 0.005 | NS | 0.013 |
| PL | NS | 0.008 | NS | NS | NS | NS |
| T | 0.005 | 0.002 | 0.005 | 0.045 | NS | NS |
| Mixed staining | | | | | | |
| SM | 0.013 | NS | NS | 0.005 | NS | NS |
| PR | 0.002 | 0.02 | NS | NS | NS | NS |
| SL | 0.005 | NS | NS | NS | NS | NS |
| Z | NS | 0.002 | 0.005 | 0.005 | NS | NS |
| PL | NS | 0.002 | 0.008 | NS | NS | NS |
| T | 0.008 | 0.002 | 0.03 | 0.013 | 0.02 | NS |
| AB staining | | | | | | |
| SM | NS | 0.004 | NS | NS | NS | NS |
| PR | 0.008 | NS | 0.005 | 0.002 | NS | 0.013 |
| SL | 0.029 | NS | 0.005 | 0.005 | NS | NS |
| Z | 0.04 | 0.004 | 0.003 | NS | 0.02 | NS |
| PL | NS | 0.008 | 0.005 | NS | NS | NS |
| T | NS | 0.012 | 0.026 | NS | NS | NS |

Between glands in dogs below the shaded boxes; between glands in cats above the shaded boxes; between cats and dogs for each gland in the shaded boxes.

* SM: submandibular; PR: parotid; SL: sublingual; Z: zygomatic; PL: palatine; T: tongue gland. NS: not significant.

Table VII. Differences (*P* values) of staining characteristics (AB positive; mixed staining and PAPS positive) of different salivary glands stained with KOH/AB/PAPS.

| Gland* | SM | PR | SL | Z | PL | T |
|------------------------|-------|-------|-------|-------|-------|-------|
| KOH/AB staining | | | | | | |
| SM | NS | 0.005 | 0.005 | NS | NS | 0.03 |
| PR | 0.004 | NS | 0.044 | 0.008 | 0.005 | 0.044 |
| SL | 0.005 | NS | 0.005 | 0.02 | 0.008 | NS |
| Z | NS | 0.004 | 0.005 | NS | NS | NS |
| PL | NS | 0.004 | 0.005 | NS | NS | 0.03 |
| T | NS | 0.004 | 0.005 | NS | NS | 0.045 |
| Mixed staining | | | | | | |
| SM | 0.003 | 0.002 | NS | NS | 0.007 | 0.022 |
| PR | 0.004 | 0.004 | 0.012 | 0.025 | 0.019 | 0.049 |
| SL | NS | 0.004 | 0.02 | NS | NS | NS |
| Z | 0.002 | NS | 0.002 | NS | NS | NS |
| PL | NS | NS | NS | NS | NS | NS |
| T | 0.024 | NS | 0.012 | NS | NS | NS |
| PAPS staining | | | | | | |
| SM | NS | NS | NS | NS | NS | 0.008 |
| PR | 0.004 | 0.005 | NS | NS | NS | NS |
| SL | 0.008 | 0.005 | 0.004 | NS | NS | 0.021 |
| Z | NS | 0.002 | 0.002 | NS | NS | 0.017 |
| PL | NS | 0.004 | 0.004 | NS | NS | 0.008 |
| T | NS | 0.002 | 0.002 | NS | NS | 0.001 |

Between glands in dogs below the shaded boxes; between glands in cats above the shaded boxes; between cats and dogs for each gland in the shaded boxes.

* SM: submandibular; PR: parotid; SL: sublingual; Z: zygomatic; PL: palatine; T: tongue gland.
NS: not significant.

method may also be of value in assessment of salivary gland tissue in disease. Changes in histochemically demonstrable mucins have been demonstrated in diseased salivary glands by Lev and Spicer [7].

ACKNOWLEDGEMENT

Mahmut Sozmen was supported by a grant from the Turkish Higher Educational Council.

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