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Structural and morphometrical study in glandular parenchyma from alcoholic sialosis

M. Carranza[1,2], M. E. Ferraris[2], M. Galizzi[1,2]

[1]*Department of Animal Morphology, School of Biology, National University of Córdoba, Argentina;* [2]*Department of Histology and Embryology, School of Dentistry, National University of Córdoba, Argentina*

BACKGROUND: The aim of this work was to determine morphometrical changes in lingual, labial and submaxillar salivary glands from alcoholic chronics.

METHODS: Five samples of each type of gland were obtained from autopsies of chronic alcoholics and equal number of samples from individuals whose death cause was accidental (controls). Serous acini in von Ebner and submaxillar glands and mucous acini in labial and Blandin–Nühn glands were analysed. In the ductal system, intra and interlobular striated ducts were studied. A digital-image analyser was used to measure different parameters in the acini and ducts.

RESULTS: Statistical analysis revealed that lingual and submaxillar salivary glands presented a significant acinar hypertrophy and hyperplasy. These changes were not observed in labial salivary glands. All studied glands showed significant structural modifications in the striated ducts.

CONCLUSION: These results indicate that the significant variations together with the histological qualitative pattern could be useful as confident indicators for the differential histopathological diagnosis with other sialosis from different aetiology.

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Introduction

Chronic alcohol consumption (CAC) produces morphological and functional alterations in different tissues and organs of the body (1). Chronic ingestion of ethanol may also cause important changes in the oral mucosae and in major and minor salivary glands. Sialosis is a salivary gland pathology associated to CAC. It is clinically characterized by a bilateral enlargement (non-neoplasic or inflammatory origin) (2–4) affecting mainly the parotid gland and slightly the other major and minor salivary glands (5–9).

Endocrine alterations such as bulimia nervosa or hormonal disorders, have also been associated with sialosis. It has been suggested that the common aetiological cause is a neuropathy of the autonomic nervous system. The normal glandular function (salivary flow) depends on sympathetic and parasympathetic innervation (10–14).

A disturbance of the autonomic neuroregulation of acinar cells could lead to inhibition in protein secretion and/or to excessive stimulation of protein synthesis. This dysfunction would be associated with accumulation of secretory granules and consequently result in acinar hypertrophy (2, 5, 6, 11, 15).

This acinar enlargement would be one of the factors for glandular swelling in chronic alcoholics (7, 11, 16). Mandel and Hamele-Bena (11) found voluminous acini with a diameter of 100 microns in parotids from alcoholic patients while in normal patients the acinar diameters measured 40 microns (17, 18). Other authors (16) showed that the acinar diameters in alcoholic parotid gland presented mean values of 76 microns. These values were significantly different from the ones obtained for normal acini (54 microns).

In a qualitative analysis, Ferraris et al. (5, 6) and Carda et al. (15) showed histological and immunohistochemical alterations in minor salivary glands from individuals that had died of alcoholic cirrhosis. They found both, histochemical and immunohistochemical heterogeneous reactions in the alcoholics acini when compared with the controls. They also described other changes such as, modifications in the cytoskeleton of acinar cells shown by cytokeratin expression, acinar hypertrophy with atypical granular accumulation in serous acini, hyperplasy of striated ducts, enlargement of excretory terminal ducts and epithelial atrophy. These findings were associated with changes in the transcriptional activity of the acinar and ductal cells detected by AgNORs technique (9).

Correspondence: Dr M. E. Gómez de Ferraris, 25 de mayo 366. 1°P, 5000. Córdoba, Argentina. Tel.: +54 351 423 6466. E-mail: melsa@odo.unc.edu.ar
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These data suggest that there are differences in the dimensions and the number of the acinar and ductal structures of salivary glands between individuals who died of alcoholic cirrhosis and those without history of CAC.

In order to confirm or rectify the histopathological qualitative pattern that we described in previous works (6, 19), we carried out a morphometrical analysis to measure the acinar and ductal structures of the parenchyma of lingual (Blandin–Nühn and von Ebner), labial and submaxillar salivary glands from chronic alcoholic individuals that had died of hepatic cirrhosis.

Materials and methods

Five samples of each of the following salivary glands were studied: mucous lingual gland (Blandin–Nühm), serous lingual gland (von Ebner), labial and submaxillar glands. The material was obtained from the autopsies of male individuals who died of alcoholic hepatic cirrhosis. It was provided by the Forensic Medical Institute of Córdoba, Argentina. The same number of samples from individuals (matched by gender and age) with no record of chronic alcoholic consumption, whose death cause was accidental and had no pathologies associated with CAC were used as controls. All the samples were obtained from individuals with an age range between 40 and 65 years old.

Material was fixed in formaldehyde 10%, at pH 7 within 24 h after *post-mortem*, then dehydrated and embedded in paraffin. Serial slices of approximately 5 microns thick were stained with Masson Trichromic (varying Lillie) technique (20).

Morphometrical study was carried out in serous acini of von Ebner lingual gland and submaxillar gland. Likewise, mucous acini from Blandin–Nühm lingual gland and labial gland were analysed. As regards the ductal system we considered the intra and interlobular striated ducts. The striated ducts of the von Ebner and Blandin–Nühn glands, were analysed altogether as there are no structural or functional differences between them (21).

To obtain the mean values of the data, 70 records per parameter and per sample from each gland were analysed in randomly selected microscopic fields. Digital imaging was used to measure the parameters observed in Table 1.

The following equations were applied in order to obtain the corresponding values for the variables: area occupied by the cells in an acinus and area of one acinar cell:

Table 1 Parameters measured in each salivary gland

Serous and mucous acini	*Striated ducts*
Total acinar area	Total ductal area
Acinar longest axis	Ductal longest axis
Acinar lumen area	Ductal lumen area
Acinar cell number	

Table 2 Mean values obtained for analysed parameters in mucous acini (Blandin–Nühn glands) and serous acini (von Ebner glands) in alcoholic and control groups

Groups	*Total acinar area (μm^2)*		*Acinar longest axis (μm)*		*Acinar lumen area (μm^2)*		*Acinar cell number*		*Area occupied by the cells in an acinus (μm^2)*		*Area of one acinar cell (μm^2)*	
	Blandin–Nühn	*von Ebner*	*Blandin–Nühn*	*von Ebner*	*Blandin–Nühn*	*von Ebner*	*Blandin–Nühn*	*von Ebner*	*Blandin–Nühn*	*von Ebner*	*Blandin–Nühn*	*von Ebner*
Control (mean ± SD)	1919.49 ± 104.54	1276.63 ± 138.26	53.04 ± 5.25	47.33 ± 4.42	127.41 ± 28.54	52.58 ± 8.93	10.28 ± 0.3	9.82 ± 0.27	1729.39 ± 107.60	1198.45 ± 126.54	172.52 ± 11.93	118.49 ± 12.22
Alcoholic (mean ± SD)	3188.31 ± 90.73	2001.68 ± 194.42	66.53 ± 6.74	56.84 ± 1.92	402.85 ± 106.65	157.34 ± 20.46	12.76 ± 0.46	12.13 ± 0.40	2778.14 ± 154.24	1845.15 ± 182.44	219.66 ± 15.35	153.13 ± 15.23
P-value	0.008*	0.008*	0.008*	0.008*	0.008*	0.008*	0.008*	0.008*	0.008*	0.008*	0.008*	0.016*

*Significant differences at $P < 0.05$; $n = 5$; µm, microns.

$$\text{Area occupied by the cells in an acinus} = \text{Total acinar area} - \text{Acinar lumen area}$$

$$\text{Area of one acinar cell} = \frac{\text{Area occupied by the cells in an acinus}}{\text{Acinar cell number}}$$

In the same way, the area occupied by the ductal epithelium was calculated using the following equation:

$$\text{Area occupied by the ductal epithelium} = \text{Total ductal area} - \text{Ductal lumen area}$$

The values for the selected parameters were obtained by means of a digital-image analyser with the Optimas software (Image Analysis Software Package Optimas version 6.1©; Optimas Corporation, Bothell, WA, USA) attached to a video colour camera and an optic microscope (Axiophot Zeiss: Zeiss, Halbergmoos, Germany) using magnifications between 250 and 640×.

The mean values obtained were compared by the *U*-test (a nonparametric test for reduced number of samples) (22). The significance level was set at $P \leq 0.05$.

Results

The results obtained form digital imaging processing indicated that all analysed parameters for mucous acini of Blandin–Nühn lingual gland and serous acini of von Ebner lingual gland (Table 2; Fig. 1a,b) were significantly higher in chronic alcoholics ($P < 0.05$) than in controls.

In the same way, the total area, lumen area and longest axis of the striated ducts from these glands were larger ($P < 0.05$) in chronic alcoholics than in controls (Table 3). Although the variable area occupied by the ductal epithelium presented a similar pattern, there were no statistical differences with the control group.

Most of the parameters analysed in mucous acini of labial glands (Table 4) did not exhibit significant differences between alcoholics and controls. However, significant differences ($P < 0.05$) were observed in the acinar lumen area, which was bigger in the alcoholic group.

Significant differences in all analysed variables ($P < 0.05$) were observed in the striated ducts of labial glands (Table 3; Fig. 1c). Most of them showed higher values ($P < 0.05$) in alcoholic individuals than in controls. Only the area occupied by the ductal epithelium was significantly smaller in alcoholics.

We also found significant differences ($P < 0.05$) in all the analysed variables of serous acini from submaxillar glands (Table 4; Fig. 1d), which presented higher values in alcoholic individuals than in controls.

The total ductal area, ductal longest axis and area occupied by the ductal epithelium of striated ducts from submaxillar glands were significantly bigger ($P < 0.05$)

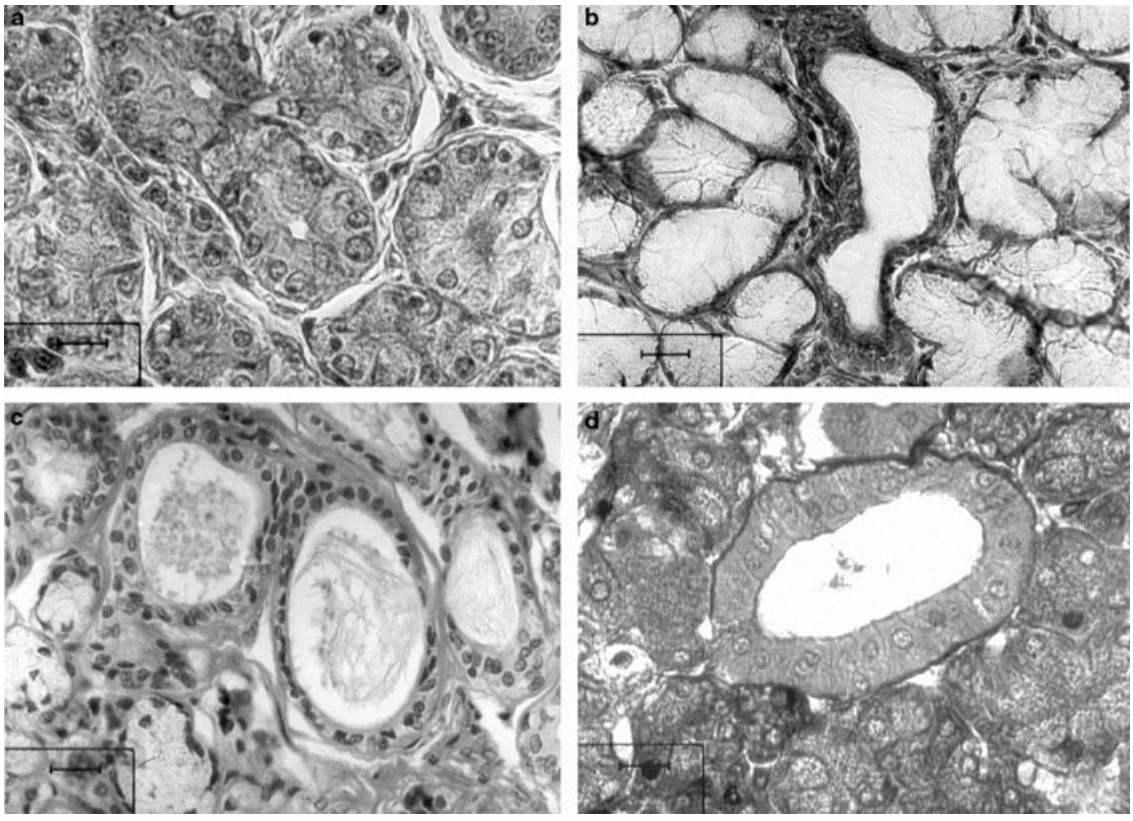

Figure 1 Alcoholic salivary glands. (a) Serous acini from von Ebner glands. Bar = 14 microns. (b) Mucous acini and duct of Blandin–Nühn gland. Bar = 28 microns. (c) Ducts with an atrophic epithelium and mucous acini from labial glands. Bar = 16 microns. (d) Duct and hypertrophic serous acini of submaxillar gland. Bar = 14 microns. Masson's Trichromic method. Bar indicates magnification power.

Table 3 Mean values obtained for analysed parameters in striated ducts of lingual, labial and submaxillar glands from chronic alcoholics and controls

	Total ductal area (μm^2)			*Ductal longest axis (μm)*			*Ductal lumen area (μm^2)*			*Area occupied by the ductal epithelium (μm^2)*		
Group	*Lingual*	*Labial*	*Subm.*	*Lingual*	*Labial*	*Subm.*	*Lingual*	*Labial*	*Subm.*	*Lingual*	*Labial*	*Subm.*
Control (mean ± SD)	2693.94 ± 392.53	3251.99 ± 182.75	2082.59 ± 120.03	69.57 ± 6.57	68.64 ± 4.12	57.82 ± 2.14	788.96 ± 127.22	622.86 ± 64.92	224.79 ± 15.22	1903.44 ± 271.21	2641.97 ± 214.25	1861.78 ± 112.57
Alcoholic (mean ± SD)	4779.45 ± 654.75	4286.58 ± 370.97	3667.45 ± 264.28	94.28 ± 4.44	89.46 ± 3.53	78.19 ± 3.68	2734.52 ± 601.42	2183.74 ± 321.53	1189.82 ± 158.73	2066.16 ± 178.13	2147.44 ± 141.75	2485.64 ± 142.34
P-value	0.008*	0.008*	0.008*	0.008*	0.008*	0.008*	0.008*	0.008*	0.008*	0.548	0.016*	0.008*

*Significant differences at $P < 0.05$; $n = 5$.
Subm., Submaxillar; μm, microns.

Table 4 Mean values obtained for analysed parameters in mucous acini of labial glands and serous acini of submaxillar glands from chronic alcoholics and controls

	Total acinar area (μm^2)		*Acinar longest axis (μm^2)*		*Acinar lumen area (μm^2)*		*Acinar cell number*		*Area occupied by the cells in an acinus (μm^2)*		*Area of one acinar cell (μm^2)*	
Group	*MALG*	*SASG*	*MALG*	*SASG*	*MALG*	*SASG*	*MALG*	*SASG*	*MALG*	*SASG*	*MALG*	*SASG*
Control (mean ± SD)	1852.89 ± 88.71	1420.92 ± 30.00	54.78 ± 2.59	45.71 ± 1.48	93.82 ± 27.71	31.87 ± 2.03	9.52 ± 0.7	10.11 ± 0.26	1763.56 ± 97.32	1387.20 ± 25.52	186.70 ± 11.36	137.92 ± 5.36
Alcoholic (mean ± SD)	2065.72 ± 200.36	2007.45 ± 121.31	56.64 ± 2.48	56.43 ± 1.27	143.98 ± 36.16	61.93 ± 9.43	11.22 ± 1.16	10.96 ± 0.51	1917.08 ± 176.42	1948.01 ± 123.45	173.79 ± 18.01	179.16 ± 16.05
P-value	0.095	0.008*	0.421	0.008*	0.032*	0.008*	0.056	0.032*	0.222	0.008*	0.421	0.008*

*Significant differences at $P < 0.05$; $n = 5$.
MALG, mucous acini of labial glands; SASG, serous acini of submaxillar glands; μm, microns.

in chronic alcoholics than in controls (Table 3). In these ducts, the ductal lumen area was significantly wider ($P < 0.05$) in alcoholic individuals.

Discussion

In this study we observed a statistically significant increment in the dimensions of the mucous and serous acini of the lingual and submaxillar glands from chronic alcoholics. Labial glands presented a similar pattern, although the size of the acini did not vary significantly between the two studied groups. Mandel and Hamele-Bena (11) demonstrated that the diameter of the serous acini of parotid gland from chronic alcoholics varies between 70 and 100 microns. In contrast, Droese (17) and Donat and Seifert (18) observed diameters of normal serous acini of 40 microns.

The average values obtained for the acinar longest axis in the von Ebner and submaxillar gland from the control group are close to the values indicated by Droese (17) and Donat and Seifert (18). In the alcoholic individuals, both glands showed serous acini of significantly larger dimensions. However, these values were similar to those referred by Gupta and Sodhani (16) for the normal serous acini. This author (16) found that the diameter of the serous acini in alcoholic parotid sialosis had increased significantly (76 microns) in comparison with the controls (54 microns).

The increment in the acinar dimensions could be related to the fact that the acini showed an increased number in cells, each one with a bigger cellular area; therefore the area occupied by all cells was more extensive. These changes could be associated with acinar hypertrophy and hyperplasy. Moreover, the presence of a significantly larger lumen in the alcoholic acini would contribute to the total enlargement of each acinus.

The mucous acini from labial glands were scarcely affected by CAC, in accordance with the results found by other authors (7). In contrast to the lingual (von Ebner and Blandin–Nühn) and submaxillar glands, the labial gland does not develop a significant hypertrophic response even in presence of terminal alcoholic hepatic cirrhosis. These results show that the acinar size of the labial gland would not be a good indicator for the differential histological diagnosis.

The striated duct system (intralobular and interlobular) of all the analysed glands was notably affected by the CAC. The total area, longest axis and lumen of all ducts were significantly bigger in the chronic alcoholic individuals than in controls. The enlarged excretory ducts could be associated with accumulation of secretory material (stasis). These observations are in accordance with the descriptions made by other authors (6, 15) in biopsies and autopsies of different glands from chronic alcoholics.

The observed reduction in the area occupied by the ductal epithelium of labial glands suggests that in alcoholic hepatic cirrhosis there is an epithelial atrophy. In lingual and submaxillar glands this alteration was not observed. These findings agree with qualitative observations from Ferraris et al. (6).

An increment above 20% was observed in most of the analysed variables in acini and ducts from alcoholic individuals. However, the labial gland exhibited smaller percentage distributions that were not significantly different ($P > 0.05$). The area occupied by the ductal epithelium in this gland presented a reduction of 18.8% ($P < 0.05$). In the lingual and submaxilar glands the area of acinar lumen increased 67%, while in the labial gland this variable reached an increment of 35%. The increment in the area of ductal lumen varied between 71 and 81% in the three studied glands.

The acinar lumen area and ductal lumen area of the von Ebner gland exhibited a slightly different response to CAC when compared with submaxillar glands. Moreover, the nucleolar organizer regions (NORs) of this minor salivary gland seem to be more affected by the chronic alcoholism than the lingual gland of Blandin–Nühn (9).

It is known that the CAC affects mainly the major salivary glands (8, 11). However, changes of diverse magnitude occur in the minor salivary glands (6, 7, 19, 23). According to our results, the glandular enlargement in alcoholic sialosis could not only be associated to abundant adipose infiltration of the stroma and slight interstitial oedema (8, 15) but also to an increment in the dimensions of ductal and acinar structures (hypertrophy) although some authors deny such possibility (24, 25).

The alcohol or its metabolites probably cause the changes in the functional units of the analysed glands. This could provoke the formation of lipid inclusions in the acinar and ductal cells as suggested by other authors (15). A disorder in the lipid metabolism caused by hepatic dysfunction would contribute to the particular glandular swelling shown in alcoholic sialosis (2, 8, 26).

The different behaviours of the studied glands would be due to diverse factors such as, their localization in the oral cavity, the reaction capacity or resistance to the cytotoxic effects of alcohol or its metabolites and/or to possible individual variations in the reactions to CAC. It has been suggested that the size of the glandular enlargement could be related to the quantity of alcohol consumed (27).

These quantitative parameters that showed significant variations, together with the histological qualitative pattern (atypical distribution of intracytoplasmic secretory vesicles in serous acini, hyperplasy of striated ducts, very enlarged terminal excretory ducts, epithelial atrophy and fatty infiltration at different levels) (5, 6, 19) are a useful tool to differentiate the alcoholic sialosis from other non-inflammatory neither neoplasic diseases of different aetiology.

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