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RESEARCH ARTICLE

Vagaries in the Labial Minor Salivary Glands Associated with Extravasation Mucoceles- A Histological and Histochemical Pilot Study of 20 cases

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ABSTRACT

Extravasation mucoceles are common in minor salivary glands of lower lip. The treatment with least recurrence rate is excision along with associated minor salivary glands; alterations of which are rarely studied. The present study attempts to identify deviations noted in labial minor salivary glands.

Aim: To analyze histological and histochemical changes in labial minor salivary gland lobules close to areas of mucin spillage and correlate clinically.

Materials and Methods: Twenty cases of labial extravasation mucoceles and their associated minor salivary glands were studied under hematoxylin-eosin and alcian blue stains.

Results: Left lower lip was commonly affected (70%). The ratio of superficial to deep extravasation phenomenon was 11:9 and 60% were in resorptive stage. The acini were predominantly mucous and 25% showed acinar degeneration. Intra-lobular ductal dilation was noted in 25% of cases. Inflammation in gland lobules was evident in all cases- 50% mild, 45% moderate and 5% dense. 60% showed congested blood capillaries and 20% interstitial fibrosis. Recurrence was principally seen in superficial mucoceles, was statistically significant at p=0.02%.

Conclusions: Alcian blue stain adds value in detecting the mucous cells and areas of mucin presence. Superficial mucoceles have a tendency to recur. Recurrence was common in initial lesions and when inflammation in minor salivary glands was moderate to dense; though not statistically significant. The related minor salivary glands are to be removed to avoid recurrence since they are a constant source of mucin spillage. Most of them are affected exhibiting inflammation, intra ducal mucin stalling and congested blood capillaries.

Keywords: Lower lip mucoceles, mucous extravasation phenomenon, lower lip sialadenitis, labial minor salivary gland, intraductal mucin, acinar degeneration

Introduction:

Mucoceles are the most common reactive lesions, predominantly seen in the lower lip making around 85% of all cases of mucoceles. The prevalence is around 0.08% in Brazil, 0.11% in Sweden and involves 2.5 out of every 1000 cases in America.[1-3] In one of the studies done on Indian subjects regarding the salivary gland lesions, mucoceles were the second most common salivary gland pathologies.[4] In general population, the incidence of mucoceles is 0.4% to 0.9%.[5] Mucoceles represent second most common soft tissue lesions following irritational fibromas.[6]

Mucoceles are mainly mucous extravasation cysts, occurring as a result of accumulation of mucous in the connective tissue due to severed excretory ducts. The ducts are severed either due to trauma or continued accumulation of secretions leading to ductal dilation followed by duct severance.[7] Salivary duct cyst or sialocyst is the term commonly used for mucous retention phenomenon that results from accumulation of mucous in the salivary gland ducts either due to a sialolith or strictures.[3,8-10]

Extravasation mucoceles are largely seen under 30 years of age whereas retention cysts is predominant in older age group.[1] Clinical appearance of both extravasation and retention cyst is the same. They appear as bluish swelling with a thin mucosal covering if they are superficially placed. Deeply placed lesions show normal color as the surrounding mucosa. The lesions are soft to palpate.[5]

Despite being benign lesions, they cause discomfort and interfere with normal oral functions such as mastication, speech, swallowing, esthetics and can recur.[2] The recurrence rate ranged from 2.8% to 18% on analysis from different studies.[8] Various management options such as use of lasers, diode lasers, marsupialization, micromarsupialization, injection of sclerosing agents and photoelectron radiation have been tried for management of mucoceles. The most successful treatment with least recurrence rate has been surgical excision with removal of associated minor salivary glands (MSG).[1,2,6] Rarely have studies been done to understand the alterations noted in associated MSG which have to be removed to prevent recurrence. The present study was done to understand the change seen in MSG associated with mucous extravasation cysts. This might throw light in understanding the pathology better; and finally help us to ensure a lower recurrence rate and to reestablish better oral health. Only the cases of lower labial mucosa were chosen for the study as this was the common site.

Materials and Methods:

Previously diagnosed archival cases of 20 lower lip mucoceles- extravasation phenomenon with associated MSG were chosen for the study. The clinical details were recorded. The sections were stained with hematoxylin-eosin and alcian blue stains. The following histopathological parameters were assessed with both the stains.

1. The *location* of the extravasation phenomenon: Mucoceles were considered to be *superficial* when the areas of mucin spillage were close to the epithelium or were located in the sub-epithelial region. Any deeper mucin spillage than sub-epithelial connective tissue were considered as *deep*.

2. The *pathogenetic stage* of the mucocele: Extravasation mucoceles demonstrate 3 stages of development:

- Stage I/ Initial stage: Mucous is seen in the connective tissue, is not well circumscribed and contains few leukocytes and histiocytes.
- Stage II/ Resorption stage: Granuloma formation is noted that contains histiocytes, foamy macrophages and giant cells with intracytoplasmic mucin.
- Stage III/ End stage: Pseudocystic space is seen that is lined by connective tissue capsule consisting of fibroblasts and fibrocytes, blood capillaries, mucinophages and inflammatory cells.[11,12]

Partially organized wall of connective tissue with multiple areas of spillage of mucin not surrounded entirely by connective tissue wall were considered as resorptive stage. Only those mucoceles where the spilled mucin was completely surrounded by connective tissue wall was considered as end stage.

3. The changes seen in the associated MSG close to area of mucin spillage:

- Acini changes: Type of acini and presence/ absence of degeneration.
- Ductal changes: Presence of intraductal mucin, dilation of intralobular ducts and presence of excretory duct around the area of mucin spillage.
- Interstitial connective tissue changes: Inflammation, congestion of blood capillaries, fibrosis of the minor salivary gland lobules.

Results:

Statistical Package for Social Sciences [SPSS] for Windows Version 22.0 Released 2013. Armonk, NY: IBM Corp., was used to perform statistical analyses. Descriptive analysis of all the explanatory and outcome parameters was done using frequency. Mean and standard deviation was used for continuous variables. Categorical variables were compared using the Chi-Square Test and Fisher's exact test was used for smaller frequencies. The level of significance was set at $p<0.05$.

DEMOGRAPHIC, ETIOLOGICAL AND RECURRENCE DATA:

The study population had a mean age of 21.45 ± 10.67 years, with the youngest patient being 5 years old and the oldest 53 years old. The majority of cases were concentrated in the second (40%, n=8) and third decades (40%, n=8) of life. This indicated that lower lip mucoceles were more common in younger individuals below 3rd decade of life. There was equal gender predilection with

a ratio of 1:1. The duration of the lesion was less than a month in most cases (60%). The left lower lip (70%) was most common site. The size of the lesions varied, with the most frequent dimensions being 1 × 1 cm in 30.0% (n=6) of patients.

Lip biting was the most frequent cause, reported in 20.0% (n=4) of patients. Other contributing factors included thumb sucking, trauma, smoking, tobacco chewing, and orthodontic brackets, each accounting for 5.0% (n=1). However, in 55.0% (n=11) of patients, no specific etiological factor was identified. The lesions were mainly asymptomatic with pain and multiple bleeding spots noted in one case each. Recurrence was noted in 25% of cases
[Table 1].

Table 1: Demographic and Etiological Data

Variable	**Category**	**n**	%
Age	≤ 10 yrs.	2	10.0%
	11-20 yrs.	8	40.0%
	21-30 yrs.	8	40.0%
	> 30 yrs.	2	10.0%
		Mean	SD
	Mean	21.45	10.67
	Range	05 - 53 yrs.	
Gender	Males	10	50.0%
	Females	10	50.0%
Duration	≤ 1 month	12	60.0%
	2-3 months	7	35.0%
	5 months	1	5.0%
Site	Left Lower Lip	14	70.0%
	Right Lower Lip	6	30.0%
Size	< 1 x 1 cm	5	25.0%
	1 x 1 cm	6	30.0%
	1.5 x 1 cm	5	25.0%
	1.5 x 1.5 cm	1	5.0%
	2 x 2 cm	3	15.0%
Etiology	Lip biting	4	20.0%
	Thumb sucking	1	5.0%
	Trauma	1	5.0%
	Smoking	1	5.0%
	Tobacco Chewing	1	5.0%
	Orthodontic Brackets	1	5.0%
	No etiology	11	55.0%
Recurrence Rate	Recurred	5	25.0%
	Not Recurred	15	75.0%

HISTOPATHOLOGICAL DATA:
Around 55% (11) cases showed superficial mucin spillage close to epithelium (Figure 1a&b) and 45% (9) cases showed mucin spillage in deeper areas (Figure 1c&d).

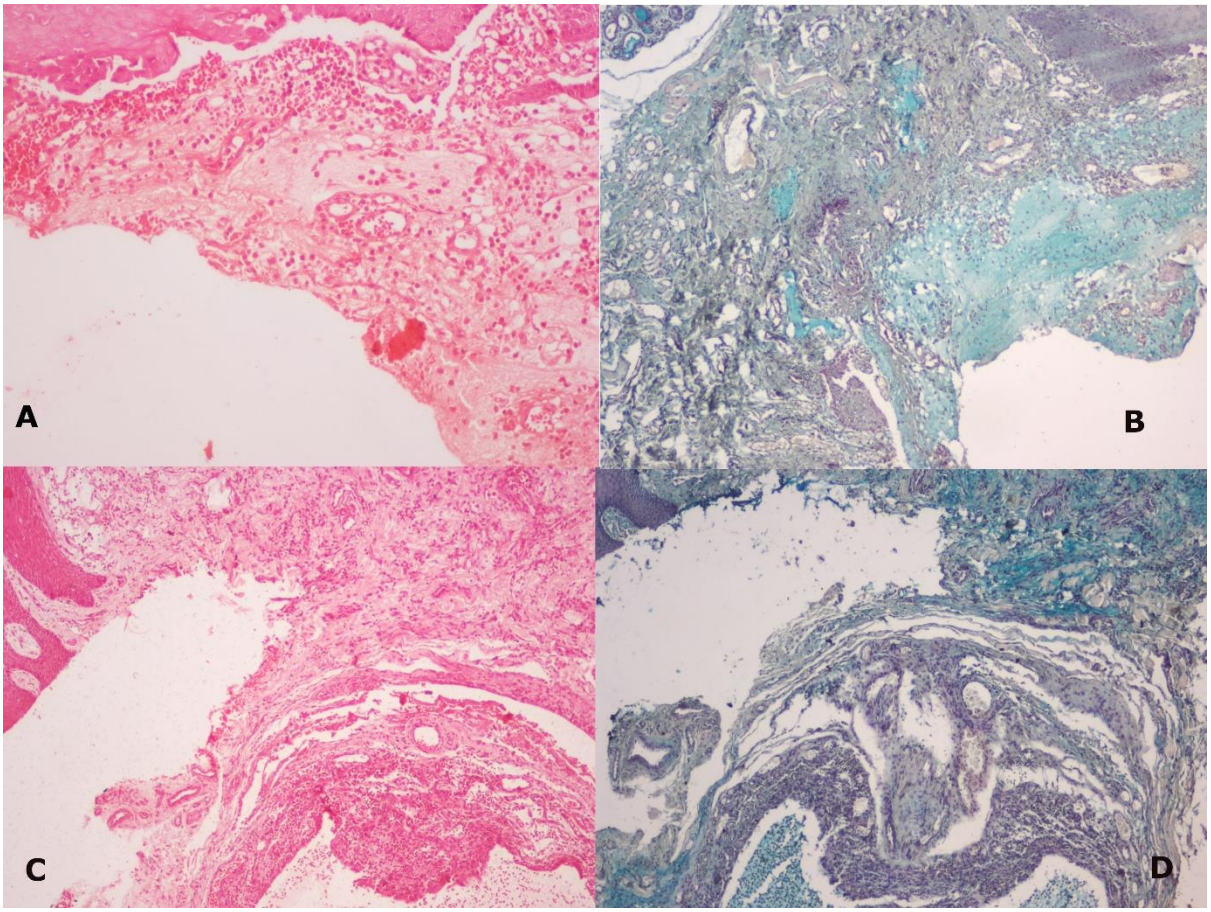

Figure 1: Superficial area of mucin spillage just below the epithelium: A] H & E stain, 40x, B] Alcian blue stain, 40x. Deep area of mucin spillage surrounded by fibrous connective tissue wall: C] H & E stain, 40x, D] Alcian blue stain, 40x.

Around 30% (6) cases were in initial stages (Figure 2a), 60% (12) cases were in resorptive phase (Figure 2b), 10% (2) cases were in end phase (Figure 2c) [Table 2]. This suggests that most mucoceles were identified in resorptive stage. Around 74% of cases shows single area of mucin spillage whereas 25% of cases showed multiple areas of mucin spillage. These areas were better detected using alcian blue stains.

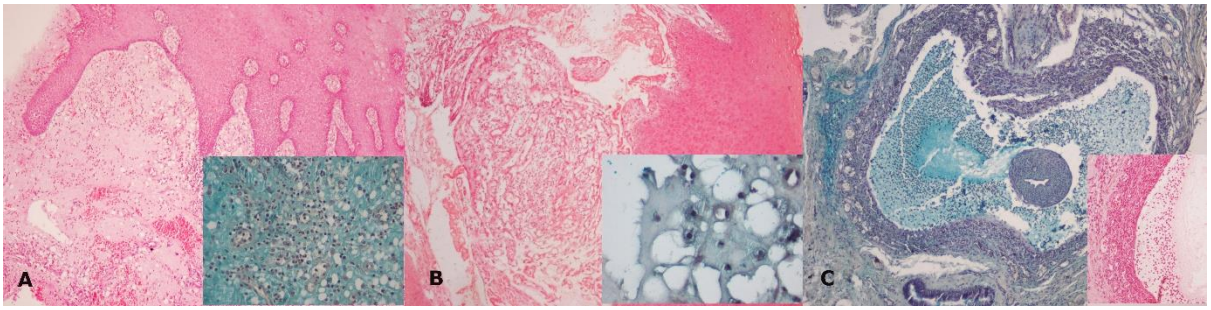

Figure 2: A] Initial stage: Mucin spillage with acute inflammatory cell response, H & E stain, 40x; Inset: Neutrophils in mucin background, Alcian blue stain, 200x. B] Resorptive stage infiltrated by chronic inflammatory cells, H & E stain, 40x; Inset: Mucinophages and few lymphocytes, Alcian blue stain, 200x. C] End stage, Alcian blue stain, 40x; Inset: Connective tissue wall surrounding spilled mucin, H & E stain, 200x.

CHANGES SEEN IN THE ACINI:

The acini were predominantly mucous in nature. This was better detected on alcian blue stain where even a small droplet of mucin was easily detected (Figure 3a & b). Few acini were closed with mucous cells polygonal in morphology rather than being low columnar. Alcian blue stain also helped to detect the free mucin in the salivary lobules (Figure 3c). Acinar degeneration was seen in 25% of cases (Figure 3d). Acini were considered to be degenerated when a lobule of salivary gland had only ductal cells and no acini. [Table 2]

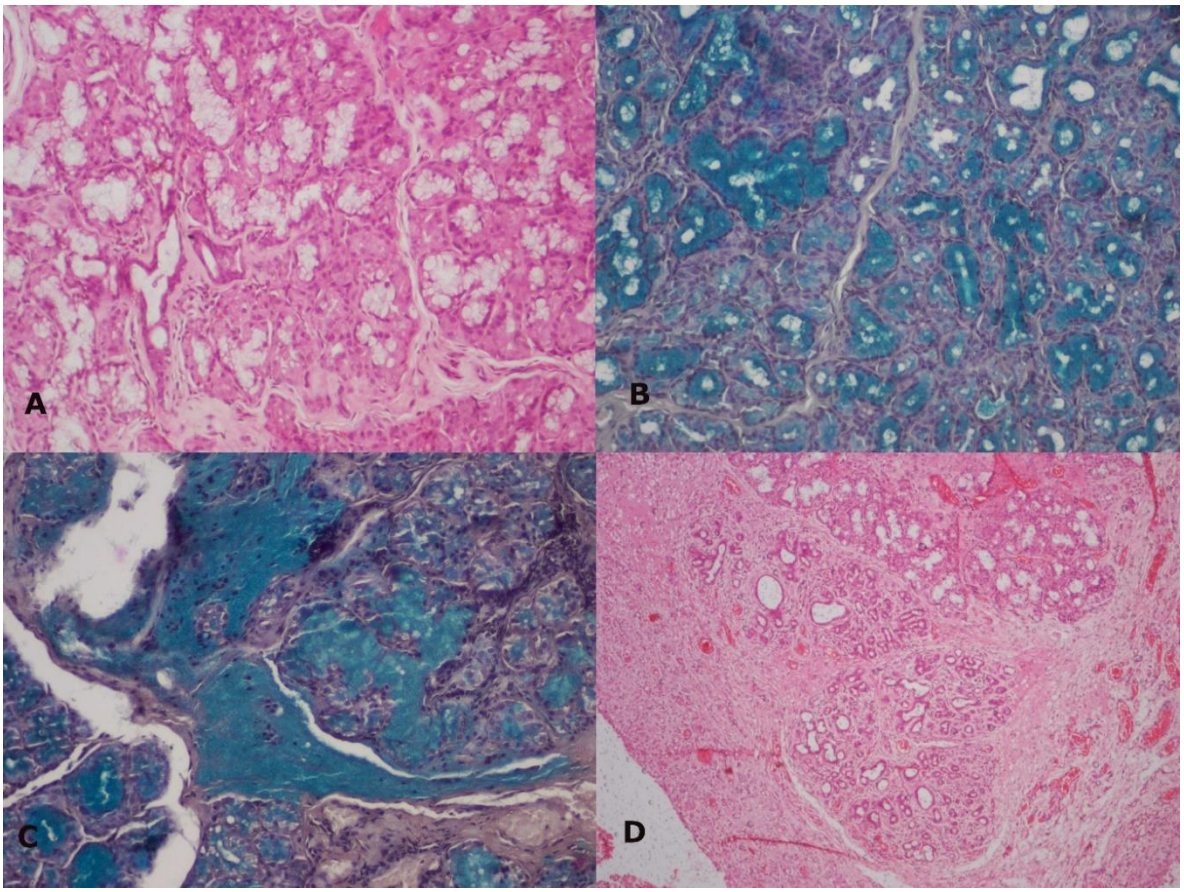

Figure 3: A] Few mucous acini with empty cytoplasm and few with eosinophilic cytoplasm, H & E stain, 40x. B] Most of the mucous acini being positive for alcian blue indicating presence of mucous droplets, Alcian blue stain, 40x. C] Areas of free mucin in salivary gland lobule, Alcian blue stain, 40x. D] Degenerating acini, H & E stain, 40x.

CHANGES IN THE DUCTS:

Presence of excretory ducts around the area of mucous expulsion was seen in 90% (18) cases and excretory duct severance was note in one case (Figure 4a). Mucin in the intralobular ducts of the salivary gland lobules were better detected with alcian blue stain and was noted in 90% (18) cases, but ductal dilation was seen in 25% (5) cases (Figure 4b). The ducts were considered to be dilated, when the lining cuboidal cells were compressed. [Table 2]

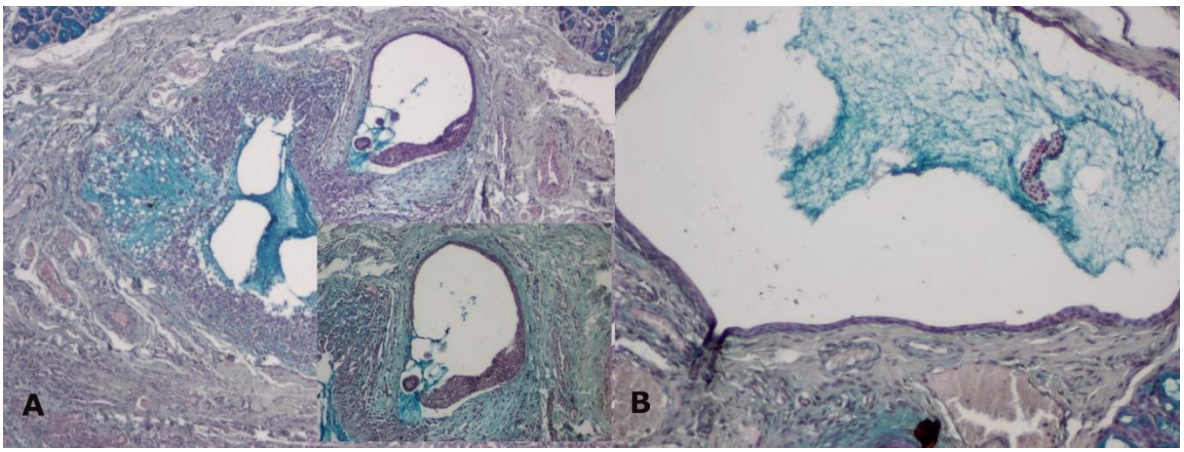

Figure 4: A] Excretory duct lined by stratified epithelium with compressed areas and duct severance. Alcian blue stain, 40x; Inset: High power view of the severed cut, Alcian blue stain, 100x. B] Intraductal mucin, ductal dilation with compression of lining cells, Alcian blue stain, 100x.

CHANGES IN THE INTERSTITIAL CONNECTIVE TISSUE OF THE SALIVARY GLAND LOBULES:

The inflammatory infiltrate (Figure 5a) noted in MSG was mild inflammation in 50% of cases, moderate in 45% and dense in 5% of cases. The inflammatory cells were predominantly lymphocytes and plasma cells; few neutrophils and eosinophils were also seen. The density of inflammatory cells was noted excluding the duct associated lymphoid tissue which is normally found around the ducts. Interstitial fibrosis was seen in 20% (4) cases (Figure 5b) and congested blood capillaries were seen in 60% (12) cases (Figure 5c). The interstitial connective tissue was predominantly loose (80% of cases). [Table 2]

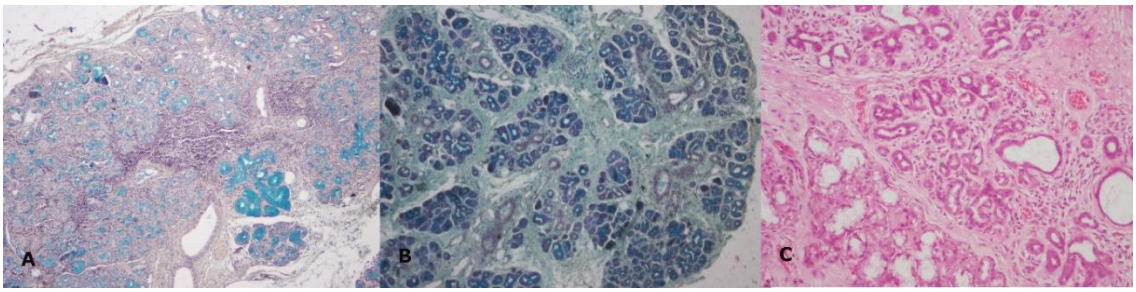

Figure 5: A] Minor salivary gland lobules showing moderate inflammatory infiltrate, Alcian blue stain, 40x. B] Fibrosis between the lobules of minor salivary gland, Alcian blue stain, 40x. C] Congested blood capillaries in and around salivary gland lobules, H & E stain, 40x.

Table 2: Distribution of Histopathological characteristics of Mucoceles among study patients

Characteristics	**Variable**	**Category**	**N**	**%**
	Location	Superficial	11	55.0%
		Deep	9	45.0%
	Stage	Initial	6	30.0%
		Resorptive	12	60.0%
		End	2	10.0%
	Areas of mucin spillage	Single	15	75.0%
		Multiple	5	25.0%
Changes in Acini	Predominant Type	Mucous	20	100.0%
	Degeneration	Yes	5	25.0%
		No	15	75.0%
Changes in the Duct	Excretory duct	Present	18	90.0%
		Absent	2	10.0%
	Intraductal mucin	Present	18	90.0%
		Absent	2	10.0%
	Ductal dilation- intralobular	Present	5	25.0%
		Absent	15	75.0%
Changes in the Interstitial connective tissue	Fibrosis	Loose CT	16	80.0%
		Fibrosis	4	20.0%
	Inflammation	Mild	10	50.0%
		Moderate	9	45.0%
		Dense	1	5.0%
	Congested BV	Present	12	60.0%
		Absent	8	40.0%

On correlating recurrent cases with histopathological presentation and features [Table 3], the following features were noted:

1. All recurrent cases n=5 (100%) were observed in superficial lesions, whereas none of the deep lesions recurred. Among the non-recurrent group, superficial lesions comprised 40.0% (n=6) and deep lesions 60.0% (n=9). This difference was statistically significant with p=0.02, indicating that superficial mucoceles were more prone to recurrence compared to deeper ones.

2. Around 60.0% (n=3) of recurrent cases were in the initial stage, 40.0% (n=2) were in the resorptive stage, and none were in the end stage. In contrast, among non-recurrent cases, 20.0% (n=3) were in the initial stage, 66.7% (n=10) were in the resorptive stage, and 13.3% (n=2) were in the end stage. Although recurrent cases tended to occur more frequently in the initial stage, the association was not statistically significant (p=0.21).

3. Degenerative changes in mucous acini were present in 20.0% (n=1) and absent in 80% (n=4) of recurrent cases. Degenerative acini was present in 26.7% (n=4) and absent in 73.3% (n=11) of non-recurrent cases. This association was not statistically significant (p=0.77).

4. Excretory duct and intraductal mucin were present in all recurrent cases 100% (n=5), whereas they were present in 86.7% (n=13) of non-recurrent cases and absent in 13.3% (n=2). These differences did not reach statistical significance (p=0.39). Similarly, ductal dilation was observed in 20.0% (n=1) of recurrent cases and 26.7% (n=4) of non-recurrent cases, with no significant association (p=0.77).

5. Inflammatory changes were mild in 40.0% (n=2) of recurrent cases, moderate in 40.0% (n=2), and dense in 20.0% (n=1). Among non-recurrent cases, 53.3% (n=8) showed mild inflammation, 46.7% (n=7) moderate, and none dense. Although moderate to dense inflammation was predominantly seen in recurrent cases, the overall association was not statistically significant (p=0.21).

6. Fibrosis in the interstitial connective tissue of MSG was observed in 20.0% each of recurrent and non-recurrent cases; likewise congested blood capillaries were seen in 60% each of recurrent and non-recurrent cases. This showed no significant difference.

Table 3: Comparison of Mucocele recurrence rate based on histopathology of MSG using Chi Square Test/Fisher Exact Test

		Recurrent Cases			Non-recurrent cases		
Characteristics	**Variable**	**Category**	**N**	**%**	**N**	**%**	**Category**
	Location	Superficial	5	100.0%	6	40.0%	0.02*
		Deep	0	0.0%	9	60.0%	
	Stage	Initial	3	60.0%	3	20.0%	0.21
		Resorptive	2	40.0%	10	66.7%	
		End	0	0.0%	2	13.3%	
	Areas of mucin spillage	Single	3	60.0%	12	80.0%	0.37
		Multiple	2	40.0%	3	20.0%	
Changes in Acini	Type	Mucous	5	100.0%	15	100.0%	..
	Degeneration	Yes	1	20.0%	4	26.7%	0.77
		No	4	80.0%	11	73.3%	
Changes in the Duct	Excretory duct	Present	5	100.0%	13	86.7%	0.39
		Absent	0	0.0%	2	13.3%	
	Intraductal mucin	Present	5	100.0%	13	86.7%	0.39
		Absent	0	0.0%	2	13.3%	
	Ductal dilation- intralobular	Present	1	20.0%	4	26.7%	0.77
		Absent	4	80.0%	11	73.3%	
Changes in the Interstitial connective tissue	Fibrosis	Loose CT	4	80.0%	12	80.0%	1.00
		Fibrosis	1	20.0%	3	20.0%	
	Inflammation	Mild	2	40.0%	8	53.3%	0.21
		Moderate	2	40.0%	7	46.7%	
		Dense	1	20.0%	0	0.0%	
	Congested BV	Present	3	60.0%	9	60.0%	1.00
		Absent	2	40.0%	6	40.0%	

Discussion:

Extravasation mucoceles are simple reactive lesions which are not often very painful, but cause discomfort and have a tendency to recur. The treatment with good success rate without recurrence has been with excision of the lesion along with associated adjacent MSG. This prompts us to understand the changes which are noticed in these MSG lobules that direct their removal. The present study was done to detect the changes seen in the adjacent MSG associated with mucous extravasation phenomenon.

Lower lip mucoceles are more common in younger age group[13] and the present study also shows a predilection in younger age group with a median age of 21.45 ± 10.67 years. Equal gender predilection was noted similar to report by Laller et al.[14] The studies done by Sathiyamoorthy et al, Pandiar et al have found a male predilection[15,16] whereas study by Bazerra et al, Miranda et al found a female predilection.[8,12]

The most common site of occurrence of mucoceles in the current study was left lower lip (70%). This correlates with the findings of Pandiar et al who also found left lower lip to be a common site for mucous extravasation phenomenon.[16] The suggested reasons for this common site is repeated trauma due to mastication and lip biting habits and increased density of minor salivary glands in lower lip than other sites.[17] Right handed individuals have a habit of using their left side (lip biting on left side) subconsciously that might be the reason for left side to be commonly affected.[16]

Trauma to the labial minor salivary glands is considered the commonest cause for mucoceles.[18] Less than half of the present study cases (9 cases) presented with some trauma or irritation to the lower lip, but not all cases were related to trauma as identified in the current study (55% of cases). This correlates with the observations made by Bezerra et al.[12] This points to hypothese other etiologies for mucoceles. Increased ductal pressure due to mucous

plugs in the duct resulting in severance of the duct; and obstruction of the duct due to periductal chronic inflammation resulting in increased intraductal pressure and subsequent rupture of the duct are other proposed causes.[12,20,21] Radiation to the lower lip also results in formation of mucoceles due to damage to the minor salivary glands.[8] In the present study, intraductal mucin that might result in mucin plugs were seen in 90% of cases. Inflammation in the MSG were scattered in the lobules rather than being concentrated only around the ducts. None of the patients in the current study were exposed to radiations.

The histopathological features collectively indicated that lower lip extravasation mucoceles were predominantly superficial (55% cases) as against the study by Miranda[8] et al who found only 2.1% to be superficial mucoceles and Chi et al[22] who reported only 0.2% of superficial mucoceles confirmed by histopathology. Single area of mucin spillage was evident in 75% cases.

The pathogenetic stage of mucoceles depends upon amount of extravasated mucin, intensity of secretory stimulus, and the speed at which the mucus is phagocytosed by the granulation tissue.[11] In the present study, 30% of cases were in initial stage, 60% in resorptive and 10% in end stage. Indicating that most mucoceles were biopsied at resorptive stage. The end stage mucoceles (100%) showed single area of mucin spillage.

Generally, the most common change noted in MSG lobules of the present study were presence of congested blood capillaries (60% cases); inflammation (50% mild, 45% moderate, 5% dense); acinar degeneration (25% cases), intralobular ductal dilation (25% cases), and interstitial fibrosis (20% cases).

As per Gnepp DR et al, inflammation is noted in the adjacent minor salivary glands.[10,20] Many case reports have shown presence of chronic inflammation and dilatation of the duct in the associated MSG.[23-25] The adjacent salivary gland tissue also shows acinar degeneration and interstitial fibrosis as per Regezi JA et al.[26] The current literature mentions the changes noted in the MSG associated with extravasation mucoceles, but the predominant association was rarely studied.

The suggested reasons for recurrence of lower lip mucoceles are repeated trauma; sutures placed deeply leading to damage and obstruction of the duct of adjacent glandular tissue; incomplete removal of adjacent minor salivary glands; spillage of cystic content; and presence of feeding duct that was not removed.[5,6,11,18,19,25,27] The other causes for recurrence include repeated erosion and re-epithelialization that may damage the minor salivary gland duct resulting in mucous extravasation. Repeated inflammatory process might cause obstruction of the MSG duct or rupture of the duct, resulting mainly in superficial mucoceles.[21] Such situations arise in oral lichen planus, lichenoid reactions or graft versus host reactions and mucous membrane pemphigoid.[28] Literature has reported nine such associated cases. Inflammation around ducts in minor salivary glands would activate the T-lymphocytes that would target the ductal epithelial antigen, resulting in ductal cell damage and mucous extravasation.[12,16,21]

In the present study, recurrence was noted in superficial mucoceles (100%, n=5) and was statistically significant with p=0.02. This suggests repeated trauma to the superficial structures might result in recurrence of mucoceles.

Recurrent lesion mainly presented at initial stage (40% of recurrent cases), showed presence of intra-ducal mucin (100% of recurrent cases) and moderate to dense inflammation (40% moderate, 20% dense among recurrent cases). These differences did not reach statistical significance.

It has been suggested that mucoceles resolve once the acini associated with mucin spillage degenerate either due to enzyme action or action of macrophages.[30] Degenerative changes in mucous acini were present in 20.0% (n=1) and absent in 80% (n=4) of recurrent cases of the current study. Degenerative acini was present in 26.7% (n=4) and absent in 73.3% (n=11) of non-recurrent cases. Although, this association was not statistically significant (p=0.77), it suggests that degenerative changes are associated more with non-recurrent mucoceles

Overall, recurrence of mucoceles was significantly associated only with superficial location (p=0.02), while other histopathological characteristics such as stage, mucin spillage, acinar degeneration, ductal changes, connective tissue alterations, inflammation, and vascular congestion did not demonstrate statistically significant associations.

The labial minor salivary glands were predominantly mucous 20/20, and were seen in various stages of mucous secretion. This was confirmed with alcian blue stain. This correlates with the recent study done by Shen et al who found that the lower labial salivary glands were predominantly mucous in their secretion.[31]

Conclusion:

Trauma may not be the commonest cause of lower lip mucoceles. When a history of trauma is not elicited; other causes that result in inflammation in superficial epithelium, increased mucous plug formation, should be considered. Recurrent mucoceles were mainly superficial in location in the current study. Histopathological changes are noted in the associated minor salivary gland which predominantly include inflammation; congestion in blood capillaries; intralobular intraductal mucin that might result in mucin plug formation; ductal dilation; and degeneration of the acini. Use of histochemical stains such as alcian blue detects even the small droplet of mucous in the acini, areas of free mucin in the acini and also demarcates the areas of extravasated mucin. The recurrence rate can be reduced by adequate removal of these associated minor salivary gland lobules which are affected by inflammation mainly and are a continuous source of mucus extravasation.

Conflicts of Interest Statement- The authors have no conflicts of interest to declare

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