

The relation of the clinical picture to the histopathology of snuff dipper's lesions in a Swedish population

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Abstract. The relation of the clinical picture of snuff dipper's lesions to the histopathological appearance was studied in 114 male dippers aged 20-88 years.

Histological study revealed increased epithelial thickness, a vacuolated surface layer with wavelike, eosinophilic spikes directed toward the surface having a narrow, eosinophilic band as a demarcation toward the prickle cell layer, acanthosis, and a slight inflammatory reaction. Thirteen cases showed an increased number of eosinophilic granulocytes, and nine cases contained amorphous, weakly eosinophilic areas in the connective tissue papillae. No epithelial dysplasia was observed.

Increased epithelial thickness, especially the presence of a vacuolated surface layer, was the only histologic feature that could be correlated with the severity of clinical appearance of the lesions. Deeply located changes, like inflammation and amorphous areas, however, were not reflected in the clinical grading of the lesions.

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A specific, well recognized mucosal reaction is caused by snuff dipping. Studies on the histopathology of this lesion have been carried out in the United States (Smith et al. 1970, Smith 1972), South Africa (van Wyk 1965) and Denmark (Pindborg & Renstrup 1963, Roed-Petersen & Pindborg 1973). These reports are largely confirmative. However, those originating from the United States and South Africa describe the surface layer of the lesion as either ortho- or parakeratinized, whereas the Danish studies emphasize the presence of a nonkeratinized and vacuolated surface layer.

The question of a possible carcinogenicity of snuff has received special attention,

but opinions on this matter are contradictory. Some clinical reports from the United States suggest a positive correlation between oral cancer and snuff dipping (Rosenfield & Calloway 1963, Brown et al. 1965), while other studies reveal no such correlation (Wynder et al. 1957a, Smith et al. 1970, Smith 1972, 1973, 1975). Reports from Sweden are also somewhat ambiguous in this respect (Ahlbom 1937, Wynder et al. 1957b, Einhorn & Wersäll 1967). In studies where the histopathology of snuff dipper's lesions has been investigated, findings of premalignant or malignant changes are rare. In 12 specimens from 12 snuff dippers in the study by Pindborg & Renstrup (1963), and in 157 biopsies taken

from clinically severe cases among a total of 15,000 snuff dippers in the United States (Smith 1972) no epithelial dysplasia or carcinoma was found. In the study from South Africa (van Wyk 1965), four cases of "disquiet epithelium" were seen among 25 biopsies. In the most recent Danish study (Roed-Petersen & Pindborg 1973), one case of dysplasia and one of carcinoma were seen among 32 biopsies from an equal number of snuff dippers referred to a hospital clinic.

The reported differences in the tissue reaction pattern in various parts of the world, as well as differences in the tendency to malignant transformation, may link with inconsistent criteria used in the diagnosis of the lesions, dissimilar dipping habits and/or variations of snuff composition. The aim of the present investigation was to study the histopathological appearance of snuff dipper's lesions in material collected in a Swedish population. The intent was further to assess whether there was any correlation between the clinical and the histological pathology.

Materials and Methods

The material for this study was selected from an epidemiological survey of oral mucosal lesions in Sweden (Axéll 1975). Among 20,000 individuals examined, around

1,200 snuff dippers were found, and of these, 114 underwent biopsy.

On the basis of information obtained from questionnaires, the snuff dipping habits of the 114 individuals, who were all males, are characterized as shown in Tables 1 and 2.

The clinical diagnosis of snuff dipper's lesion was determined when there was a lesion of the oral mucosa in a location which, upon direct questioning, was found to be at the exact site for the regular placing of snuff. The clinical appearance of the lesions was graded as follows (Fig. 1):

Degree 1.

A superficial lesion with a color similar to the surrounding mucosa, and with slight wrinkling. No obvious mucosal thickening.

Degree 2.

A superficial, whitish or yellowish lesion with wrinkling. No obvious thickening.

Degree 3.

A whitish-yellowish to brown, wrinkled lesion with intervening furrows of normal mucosal color. Obvious thickening.

Degree 4.

A marked, white-yellowish to brown and heavily wrinkled

Table 1. Snuff habits related to clinical grading of the oral lesions

Clinical degree	No.	Age years (\bar{x})	Amount of snuff h/day (\bar{x}) g/day (\bar{x})		Duration years (\bar{x})
X	6	41.7	4.3	11.3	12.0
1	4	56.0	2.5	5.0	11.0
2	17	55.5	6.1	11.1	30.1
3	51	47.4	7.4	12.9	20.7
4	36	53.4	8.2	18.0	28.6
Total	114	50.4	6.8	13.8	23.8
Range		20-88	1-24	4-50	1-68

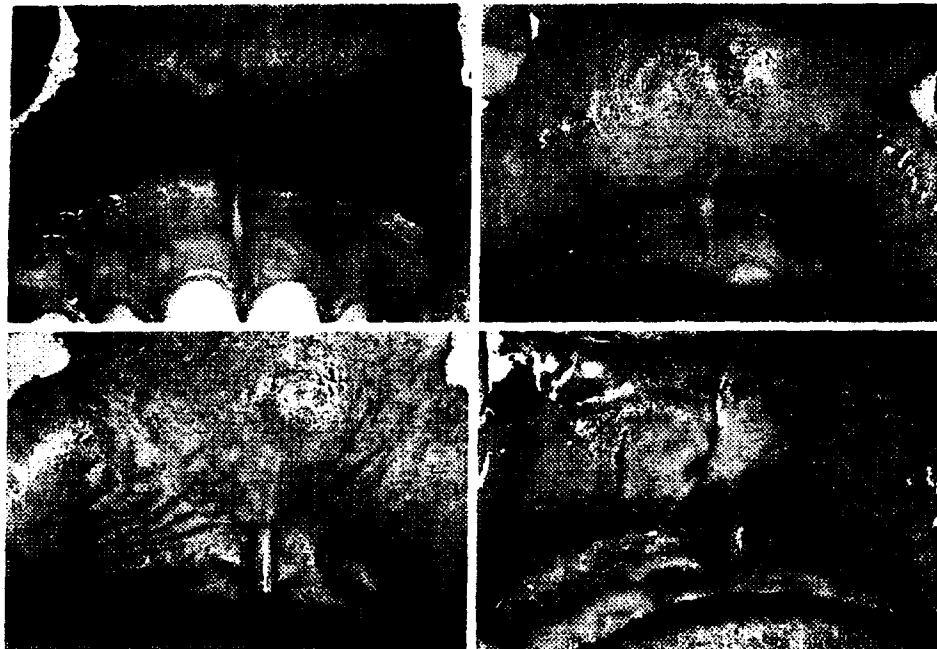


Fig. 1. Snuff dipper's lesions of clinical degrees 1-4, a-d respectively.

lesion with intervening, deep and reddened furrows and/or a heavy thickening.

4 were subjected to biopsy whereas individuals with any of the other three degrees were biopsied at random. About 90 % of the patients accepted biopsy, which was undertaken a few days after the

All patients with clinical lesions of degree

Table 2. Snuff habits related to brands of snuff

Brand of snuff	No.	Age years (\bar{x})	Amount of snuff h/day (\bar{x}) g/day (\bar{x})		Duration years (\bar{x})
I	74	54.0	7.5	14.6	27.8
II	26	46.2	6.5	11.5	18.2
III	12	39.4	3.6	11.6	14.3
IV	2	29.5	4.5	21.0	7.0
Total	114	50.4	6.8	13.8	23.8
Range		20-88	1-24	4-50	1-68

Brand I = Ettan; brand II = Grovsnus; brand III = Röda lacket; brand IV = Svenskt exportsnus.

Fig. 2. Typical appearance of snuff dipper's lesion, showing a vacuolated epithelial surface layer, which contains wave-like, eosinophilic spikes and which is demarcated from the underlying, acanthotic prickle cell layer by a narrow band of increased eosinophilic staining. Slight inflammation. H & E, $\times 108$.



screening examination. On the day of biopsy, the patient was checked for any change in his snuff habit. Some patients had stopped snuff dipping between the two examinations and were then placed in a special group called "degree X". The biopsies comprised upper or lower anterior vestibular mucosa and were taken from the central parts of the lesions with a 5.5 mm punch instrument under local anesthesia with 3 % Citanest-Exadrin®. Six biopsies were taken from clinical lesions of degree X, four from degree 1, 17 from degree 2, 51 from degree 3 and 36 from degree 4. The specimens were fixed in 10 % neutral formalin and embedded in paraffin. Sections were stained with hematoxylin-eosin, periodic acid-Schiff and van Gieson. Histological features were assessed through a subjective grading (slight, moderate, marked).

Results

All but one biopsy showed an increased total epithelial thickness, and this increase was more pronounced in the lesions clinically assigned degrees 3 and 4. In the vast majority of the lesions, the outer surface

of the epithelium consisted of a vacuolated layer, occasionally containing remnants of cell nuclei. Within this layer, wavelike eosinophilic spikes directed toward the surface were often seen, and the layer as such was usually demarcated from the cells of the underlying prickle cell layer by a narrow, often distinct, eosinophilic band (Fig. 2). This surface layer was significantly more pronounced in lesions of degrees 3 and, especially, 4 as compared to those of degrees 1 and 2.

In 22 cases, ortho- and/or para-keratinized surface layers were found in parts of the biopsies, and this feature was most often seen in lesions of degrees X, 1 and 2. In regions with orthokeratinization, a granular cell layer was usually also recognized (Fig. 3). Some of the keratinized surfaces were swollen and of a granular texture, and thus appeared as intermediate forms between undisturbed keratinization and the vacuolization described above (Fig. 4). The total epithelial thickness in keratinized areas was often slight compared to those areas in the same specimens which showed a vacuolated surface layer.

Acanthosis was found in all clinical

Fig. 3. Snuff dipper's lesion showing the presence of a granular cell layer and a slightly vacuolated, orthokeratinized surface.
H & E, $\times 108$.



groups, being somewhat marked in degrees 3 and 4. The increase of total epithelial thickness mentioned above was mainly due to the presence of the vacuolated surface layer, and only to a lesser degree to acanthosis. A slightly club-shaped epithelial hyperplasia was noted in 30 cases, without correlation to clinical severity.

None of the 114 biopsies showed changes interpreted as cellular atypia or epithelial dysplasia.

Most of the biopsies showed a very slight inflammatory reaction, and there was no correlation between the degree of inflammation and the clinical grading. Sixteen cases were characterized as mo-



Fig. 4. Lesion with surface characteristics intermediate between vacuolization and orthokeratinization.
H & E, $\times 195$.

Fig. 5. Lesion showing amorphous areas of various extensions in the connective tissue papillae. H & E, $\times 120$.



derately, and 11 cases as severely inflamed. The inflammation was often localized to the juxtaepithelial part of the lamina propria. Spongiosis and inflammatory cell infiltration of the basal layers of the epithelium was noted in cases of severe inflammation.

The inflammatory cells were predominantly lymphocytes, but plasma cells and neutrophilic granulocytes were also occasionally present. Thirteen cases showed a remarkable number of eosinophilic granulocytes, and most of these were associated with moderate or severe inflammation. In nine cases, amorphous, weakly eosinophilic, PAS-positive and van Gieson yellow areas were observed in the connective tissue papillae (Fig. 5). In one of these cases a similar and very pronounced change was also seen around a salivary duct deep in the connective tissue. Special staining of the amorphous areas with the thioflavine-T and Congo red techniques did not indicate the presence of amyloid. PTAH staining for fibrinoid also gave negative results. There was no correlation between the amorphous areas and the clinical grading. Six of the nine cases be-

longed to those with moderate or severe inflammation, and five to those with increased numbers of eosinophilic granulocytes.

The histopathological appearance of the six biopsies taken from clinical lesions of degree X differed in some respects from the other lesions. The most obvious difference was an almost total absence of a vacuolated surface. Three cases showed a thin surface layer of para- or orthokeratinization.

With one exception, there was no correlation between either the clinical or the histological appearance and the different brands of snuff used. All of the nine cases containing amorphous areas in the connective tissue belonged to the 86 biopsies taken from individuals using brands I and III. No such case was seen among the biopsies from the 28 individuals using brands II and IV.

Discussion

The most striking and consistent histological finding in the present biopsies of snuff dipper's lesions was an increased total

thickness of the epithelium. This increased thickness was due partly to the formation of a vacuolated surface layer and partly to acanthosis. The correlation found between the clinically and the histologically observed thickness of the lesions was related to the vacuolated layer, rather than to the acanthosis. The presence of a thick, vacuolated layer with eosinophilic spikes and bands, was especially emphasized by Roed-Petersen & Pindborg (1973). It seems reasonable to assume that this layer represents a direct tissue damage, probably related to the high alkaline reaction (approx. pH 9) of Scandinavian wet snuff. This assumption is supported by our finding that cessation of snuff dipping for a few days results in regression of the lesions, with loss of the vacuolated layer. Furthermore, the reports from the United States and South Africa, which do not emphasize vacuolization, relate to the use of dry, weakly alkaline snuff.

The histological assessment of inflammation showed no correlation with the clinical degree of the lesions. In cases with moderate or severe inflammation, an increased number of eosinophilic granulocytes were often found. The presence of eosinophils is not mentioned in earlier studies of snuff dipper's lesions. Whether this finding is of any specific significance in terms of immunological reactions is unknown.

Lyon et al. (1964) specifically studied deposits of an amorphous material, interpreted as amyloid, around accessory salivary glands in four of seven biopsies from snuff dipper's lesions. Archard & Tarpley (1972) found similar deposits, but did not find them to be amyloid in nature. Our biopsies were generally shallow and only occasionally contained salivary gland tissue. One of these specimens displayed an amorphous, weakly eosinophilic, PAS-positive and van Gieson yellow area around a salivary gland duct. Similar amorphous

changes were however found in eight further cases in the connective tissue papillae just underneath a thin epithelial covering. With methods employed in this investigation amyloid could not be identified. It may be assumed that the nearness of the quid of snuff to the connective tissue plays a decisive role in the etiology of this change, and that it reflects a direct chemical and/or mechanical injury. The pathogenesis should then be identical with the one earlier indicated for the vacuolated surface layer of the epithelium.

The amorphous areas in this material frequently occurred together with eosinophilic granulocytes and this may indicate a possible involvement of immunological reactions. Hamner et al. (1971) described the presence of juxtaepithelial amorphous deposits in 54 oral biopsies from patients with submucous fibrosis. Although these deposits may be specific for submucous fibrosis, it is still of interest to note that these authors discussed, among other factors, the possible etiologic role of panquids (containing tobacco and lime) and of chili. They also reported the presence of eosinophilic granulocytes and discussed the possibility of allergic reactions. The negative findings with respect to staining for fibrinoid, lessen the likelihood that the amorphous changes in our material are of immunological origin, and it may well be that the amorphous areas reflect collagen which has been altered upon a direct chemical insult.

The fact that all cases with amorphous changes were found among individuals using two particular brands of snuff (I and III) may indicate that these two brands contain substances of specific importance for the development of these changes. However, all constituents of brand I are also present in the other brands. Alternatively, the users of the two particular brands could show specific characteristics

as regards demographic data or snuff dipping habits. This hypothesis was also tested but nothing was found to support it.

None of the 114 biopsies showed cellular atypia or epithelial dysplasia. In some biopsies, epithelial changes were found which could perhaps be labelled "disquiet epithelium" (van Wyk 1965). However, as these biopsies did not show an increased number of mitotic figures but severe inflammation, these changes are interpreted as secondary to the inflammatory reaction without premalignant implications.

It appears that our clinical grading only reflects changes in the outermost layers and does not disclose any deeper tissue reactions such as inflammation or the presence of amorphous changes. The absence of epithelial dysplasia in the present material obviously excludes any statement as to whether such changes may be related to specific clinical characteristics.

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