Transferrin reactivity in oral mucosa adjacent to different dental restorations

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Syrjänen S, Syrjänen K, Yli-Urpo A. Transferrin reactivity in oral mucosa adjacent to different dental restorations. Acta Odontol Scand 1984;42:321–325. Oslo. ISSN 0001–6357.

Transferrin (TF) is a metal-binding protein that has been detected in human mucosal tissues. Positive TF reactivity has been related to iron transport, epithelial keratinization, and the non-specific defense system of mucosal membranes. An immunoperoxidase staining technique was used to study the distribution of TF in buccal mucosa adjacent to different metallic restorations (62 cases) to assess the nature of tissue changes possibly attributable to dental materials. The transferrin reactivity in the oral epithelium of 10 patients with galvanic symptoms was also determined. The results showed an obvious shift in TF reactivity from weak to strong in patients with complete dentures and in those with galvanic symptoms when compared with controls. The results are discussed in terms of epithelial cell proliferation and keratinization and of the role of TF in chelating free metallic ions. □ *Epithelial changes; immunohistochemical staining; keratinization; oral galvanism; transport of metallic ions*

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For many years metallic constituents of dental restoration have been considered safe and harmless, without any biological effects in the oral cavity. However, the vastly increased probability of electrocorrosive phenomena in the oral cavity has recently brought into focus the possible irritative effects of these materials (1–5). Recent studies have suggested that differences in electric potentials may play an etiologic role in the development of both oral leukoplakia and lichen planus (1, 2, 6–10). Hypersensitivity reactions to dental restorative materials have also been reported (2, 8), attributable to galvanic release of metallic ions (2, 8).

Transferrin (TF) is a transport protein for metals, capable of combining with iron, copper, and zinc (11, 12). The prime function of TF is to transport iron (11–13). In the skin, TF is normally present in a few epidermal cells and in dermal histocytes (14). Recently, TF reactivity was also found in the gingiva and in minor salivary glands (15, 16). The presence of TF in the skin and in oral and gastrointestinal mucosa has been related to iron transport and to non-specific defense systems at these sites (13, 14). Positive TF reactivity has also been related to the degree of cell maturation and to epithelial keratinization (14). Thus, accelerated cell proliferation and keratinization have recently been shown in the oral epithelium of patients after extensive restorative treatment and in those with galvanic symptoms (17).

The aim of the present investigation was to characterize further these epithelial changes. Special attention was focused on the role of TF in the transport of metallic ions galvanically released from the dental alloys and on its role in the proliferation of the epithelium in close proximity to the restorations involved.

Materials and methods

The series comprises a total of 110 healthy subjects divided into four groups as follows: group I, 19 patients with only a few occlusal amalgam fillings; group II, 19 patients with complete dentures; group III, 62 patients with metal restorations of different compositions; and group IV, 10 patients with clin-

Table 1. Age, male to female ratio, DMF index, and the character of the metallic restorations used in subjects studied

Group	Characterization of group	No.	Mean age (years) (M ± SD)	Male to female ratio	DMF index (M ± SD)	No. of amalgam fillings (M ± SD)	No. of gold crowns
I	A few fillings	19	34.0 ± 11.3	1:5.3	14.2 ± 3.7	8.7 ± 4.1	—
II	Complete dentures	19	63.7 ± 8.3	1:2.2	28.0 ± 0.0	—	—
III	Mixed dental metallic restorations	62	48.8 ± 1.1	1:1.2	21.0 ± 3.9	8.2 ± 3.8	4.9 ± 2.9 (53 patients)
IV	Mixed dental restorations in connection with galvanic symptoms	40	51.3 ± 9.1	1:2.5	21.6 ± 4.1	6.3 ± 4.5	14.2 ± 6.6

Table 2. Characterization of dental metallic restorations used in group III

	Total no. of restored teeth (M ± SD)	Amalgam fillings/ cobalt chromium prosthesis (M ± SD)	Amalgam fillings/ gold constructions (M ± SD)	Amalgam fillings/ gold cobalt-chromium constructions (M ± SD)
No. of subjects	62	9	42	11
Mean no. of teeth filled with amalgam	8.2 ± 3.8	6.8 ± 2.5	8.9 ± 3.4	6.8 ± 2.7
Mean no. of teeth restored with gold	4.9 ± 2.9	—	5.5 ± 3.1	3.6 ± 2.8

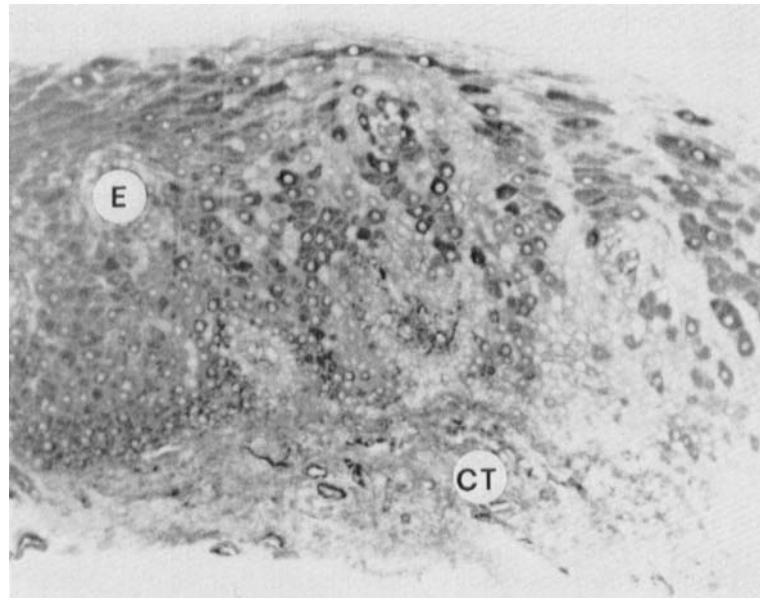

Fig. 1. Strong transferring immunocreativity in buccal epithelium (E). TF-positive cells (black) are encountered throughout the entire thickness of the epithelium (E = epithelium; CT = connecting tissue). (Immunoperoxidase kit for TF; original magnification, ×100.)

ical symptoms of oral galvanism (oral soreness, metallic taste, burning mouth).

The patients are characterized by mean ages, sex distribution, and type of dental restorations in Table 1. The metallic restorations used in group II are shown in Table 2.

All patients were subjected to a biopsy

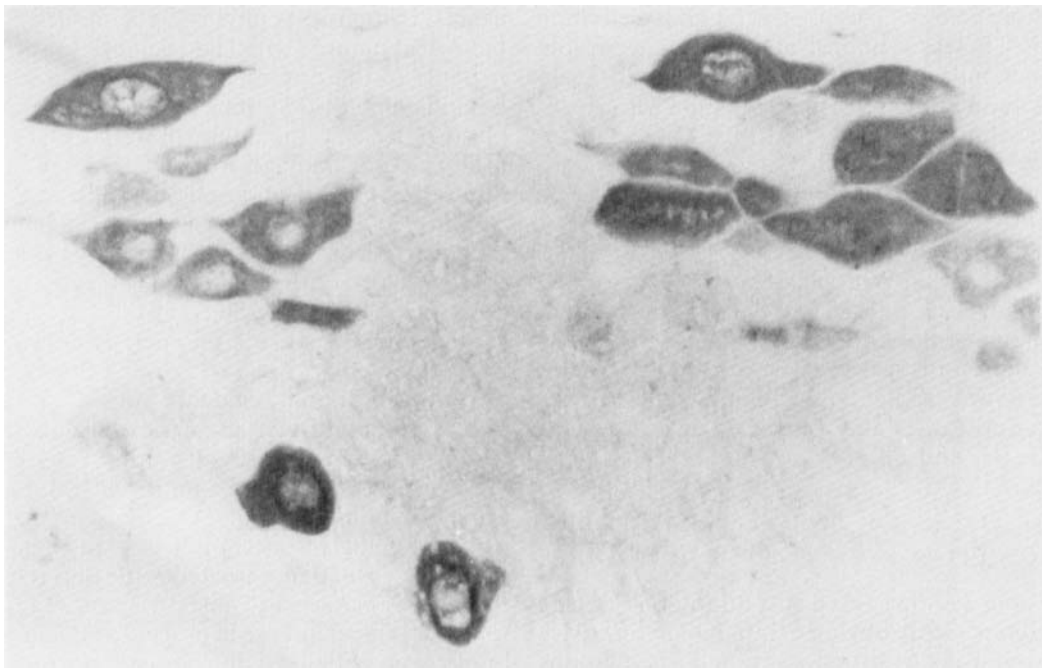

Fig. 2. High-power detail of the epithelial cells stained positive for TF. The positive staining is confined to the cytoplasm, the nucleus remaining negative. (Immunoperoxidase kit for TF; original magnification, ×400.)

Table 3. Distribution of transferrin in buccal mucosa adjacent to dental restoration materials

		Intensity of transferrin reactivity								
		Absent		Weak		Moderate		Strong		Level of significance,
Group*	No.	No.	%	No.	%	No.	%	No.	%	p<
I	19	0	0.0	8	42.1	7	36.8	4	21.1	
II	19	0	0.0	0	0.0	8	42.1	11	57.9	0.001
III	62	3	4.8	19	30.7	27	43.5	13	21.0	
IV	10	0	0.0	1	10.0	3	30.0	6	60.0	0.025

* Group I = controls; group II = patients with complete dentures; group III = patients with metal restorations of different quality; and group IV = patients with clinical symptoms of oral galvanism.

directed at the buccal mucosa in areas where two different restorative materials were in contact with each other. From control subjects (group I) the corresponding site (devoid of contact with restorations) was biopsied. In subjects with complete dentures (group II) the same site as in group I was biopsied. The tissue sample was fixed in 10% formalin and processed in accordance with routine histological procedures.

The sections (five in each specimen) were stained with hematoxylin/eosin for general morphology. The presence and distribution of TF was demonstrated in 5-μm paraffin sections by staining with commercially available immunoperoxidase kits for TF (Histoset, Immulok Inc., Calif., USA). The intensity of the positive reaction, a dark brownish-red precipitate, was classified as absent, weak, moderate, or strong (Figs. 1 and 2).

All ranking procedures were performed blind. The authors were unable to identify the patients at this stage of the work.

For statistical calculations the chi-square test was applied. Group I was used as a control series and was tested against groups II, III, and IV.

Results

Table 3 shows the distribution of TF in the sections. An obvious shift in TF reactivity from weak to strong was noted when groups II and IV were compared with controls ($p < 0.001$ and $p < 0.025$, respectively. Thus a strong reactivity for TF was obtained in 58% of denture wearers (group II) and in 60% of patients with galvanic symptoms (group IV).

Discussion

The oral mucosa is of fundamental importance in dentistry. Oral epithelium is composed of stratified squamous cells, and its function is to protect the underlying tissues against irritants of a physical, chemical, and biological nature (18). The response to such irritants of the oral mucous membrane can be inflammatory, degenerative, or hyperplastic (18). Several authors have attributed oral mucosal damage to electric potential differences between various metallic restorations (3, 7). The role of electrogalvanic microcurrents in the development of white patchy lesions of the oral mucosa is substantiated by the disappearance of these lesions subsequent to changing the metal composition (1, 9).

Some additional evidence of irritative effects of dental materials on the oral mucosa was provided by the present study, using the immunohistochemical staining method for a metal-binding protein. A distinct pattern of TF staining in buccal mucosa was demonstrated in patients treated with different restorative materials (Table 3). The staining for TF was most intense in oral mucous membrane from patients with complete dentures (group II), succeeded by patients with galvanic symptoms (group IV), and was least

intense in the controls (group I). The epithelial keratinization reportedly is most pronounced in denture wearers (17). This favors the concept that TF reactivity is related to the degree of keratinization (14, 16, 17). Increased keratinization of the oral mucosa in patients with complete dentures has been regarded as a manifestation of protective mechanisms against mechanical irritation (18). However, in group III (patients with metal restorations of different compositions), in which keratinization was recently shown in 53% of the specimens (nearly the same percentage as in group IV, patients with clinical symptoms of oral galvanism) (17), no significant difference could be found in TF reactivity when compared with controls (Table 3). The major difference in reactivity between groups III and IV in spite of equal keratinization might suggest that TF plays an important role in chelating the free metallic ions in patients with galvanic symptoms. The mode of action could be similar to that in the non-specific defense against some microorganisms (13, 19). In addition to this chelating action, the strong TF reactivity might reflect cell degeneration and release of intracellular iron picked up by TF (12).

Recently, a common TF receptor was shown on the surface of all proliferating cells (20). Whether the strong TF reactivity detected in patients with complete dentures and in those with galvanic symptoms is due to an increased number of TF receptors on these epithelial cells remains to be shown by a future study, aimed at demonstrating TF receptors immunohistochemically with monoclonal antibodies. The assessment of the relations of mucosal TF to metallic ions in saliva will, we hope, give additional information about the significance of TF in oral galvanic symptoms.

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Received for publication 22 September 1983