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Effects of zinc deficiency on oral and periodontal diseases in rats

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Background and Objective: The aim of this study was to investigate the alterations of oral and periodontal tissues in zinc-deficient rats compared with control rats.

Material and Methods: The study was carried out on 14 Sprague-Dawley rats, cessation of lactation on the 24th day after birth. Rats were randomly divided into two groups. Group I rats were fed with a zinc-deficient diet and group II rats were fed with a zinc-containing diet. At the end of the fourth week on experimental diets, alterations of the oral tissues in both groups were recorded. In addition, the gingival index (GI-Löe-Silness), plaque index (PI-Silness-Löe) and periodontal pocket depth scores were recorded in order to assess periodontal tissue health in the rats. Then, blood samples were taken and the serum zinc levels measured by atomic absorption spectrophotometry. At the end of the experiment, oral tissue samples were investigated by light microscopy. Finally, the results of the two groups were compared by using the Student's *t*-test.

Results: The effects of zinc deficiency were observed at 10–16 d in rats. Although body weight, body length and tail length were retarded in zinc-deficient rats, they were advanced in rats fed with a zinc-containing diet. The mean plaque index and gingival index for group II rats were significantly lower than for group I rats ($p < 0.001$), but there was no significant difference regarding pocket depth between the two groups of rats ($p > 0.05$). Aphthous ulcer was often seen in the study group, where it was observed on the alveolar mucosa with a high rate of 29.9%. According to histological findings, there was no difference related to the epithelial keratinization of the hard palate between the two groups. However, hyperkeratosis was found on the dorsal surface of the tongue in zinc-deficient rats.

Conclusion: The findings indicated that oral health was better in group II rats (those fed with a zinc-containing diet) than in group I (zinc-deficient) rats. Hyperkeratinization was more prominent in zinc-deficient rats. We suggest that zinc deficiency is a potential risk factor for oral and periodontal diseases.

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Zinc is known to be an essential trace element for the growth of humans and other animals (1–4). In 1958, Prasad (5) reported, for the first time, that the deficiency of zinc in humans was a syndrome characterized by growth retardation dwarfism, male hypogonadism, rough and scaly skin, loss of hair, and mental lethargy, for a patient in Shiraz, Iran. Subsequently, zinc deficiency has been reported in humans from Egypt, Turkey, Portugal, Morocco, Yugoslavia, and other developing countries (6–8). These reports represent only awareness in diagnosing overt cases, rather than the actual worldwide incidence of zinc deficiency (9).

Humans and animals experiencing zinc deficiency exhibit a wide variety of symptoms, including impaired growth, alopecia, anemia, dwarfism, impaired sexual development, geophagia, dermatitis, loss of hair, poor appetite,

abnormal dark adaptation, delayed wound healing, and mental lethargy (1–8).

The general symptoms of zinc deficiency have been reported in a number of studies (1–14); however, the effect of zinc deficiency on oral tissue has not been described in detail (15–17). In this study, we attempted to investigate the changes in the oral tissue of zinc-deficient rats and to review the related literature.

Material and methods

Study design

This investigation was carried out at the Physiology Animal Laboratory of the Medical School at Atatürk University (Erzurum, Turkey). Fourteen Sprague-Dawley rats, cessation of lactation on the 24th day after birth, were used. Rats were randomly divided into two groups: group I rats were fed with a zinc-deficient diet, and group II rats (controls) were fed with a zinc-containing diet (Table 1) (18). The zinc-deficient diet was stored at 4.5 ± 0.5°C in plastic containers and handled with plastic gloves and appropriate utensils to avoid contamination. The diet was placed in shallow glass food cups, with stainless steel follow-through disks, to reduce food spills. The rats were kept individually in stainless steel cages and maintained at 22–25°C with a 12-h light/dark cycle. They were allowed free access to double-distilled water. Features of zinc deficiency, including cutaneous lesions, loss of appetite, slowed weight gain, loss of hair, diarrhea, and alopecia, were observed in all zinc-deficient rats.

Biochemical measurements

At the end of the fourth week on experimental diets, alterations of the oral tissue in both groups were recorded. Then, all of the rats were killed by cardiac puncture after anesthesia with thiopental. Blood was removed directly from the heart. Blood samples were centrifuged at 1600 ***g*** for 5 min and maintained at −85°C until shortly before assay. The serum zinc level was measured by atomic absorption spectrophotometry (Flame type UNICAM 929; ATI-Unicam, Cambridge, UK).

Clinical evaluation

Oral manifestations related to zinc deficiency were evaluated at the end of the study. The mucosa of the palate, the floor of the mouth and the tongue, and the gum of molar and incisive teeth in the upper and lower jaws, were carefully investigated. The number of oral ulcers and their locations were recorded.

The clinical evaluation consisted of plaque index (19) and gingival index (20) scoring, and measurement of probing pocket depths. The measurements were made in the Physiology Animal Laboratory, Atatürk University, by the same periodontist.

The plaque index and gingival index scores were recorded on four tooth surfaces (mesial, distal, buccal, and lingual) for all anterior teeth, and the quantity of supragingival plaque was assessed at the cervical area of every tooth.

The numerical scores of the plaque index and gingival index were obtained according to the formula Per rat = sum of individual scores/number of anterior teeth present for each rat, and then group score was calculated by adding together the individual scores and dividing the total into the number of rats included.

The pocket depths were recorded by measuring the distance from the free gingival margin to the base of the pocket and the cemento–enamel junction with a thin wire.

Histological procedure

Tissue samples for light microscopy were fixed by immersion in 10% neutral-buffered formaldehyde for 24 h, dehydrated in a graded ethanol series, and embedded in paraffin wax. Paraffin sections, 4–5 μm thickness, were stained with haematoxylin and eosin, and examined using an Olympus BH-2 light microscope (Olympus, Tokyo, Japan).

Statistical analysis

Statistical evaluation was performed by the Mann–Whitney *U*-test. The two groups were compared using the Student's *t*-test.

Results

The investigation was carried out on 14 rats. Group I (fed with a zinc-deficient diet) consisted of seven rats (five

Table 1. Composition of a zinc-deficient diet and a zinc-containing diet (18)

		Group I	Group II
Ingredient	%	Diet (kg)	Diet (kg)
Egg white	70	0.700	0.700
DL-Methionine	1	0.010	0.010
Sucrose	12.3	0.123	0.123
Corn oil	10	0.100	0.100
Cellulose	2	0.020	0.020
Choline bitartrate	0.2	0.002	0.002
AIN-76 mineral mix[a]	3.5	0.035 (zinc deficient)	0.035
AIN-76 vitamin mix[b]	1	0.010	0.010
Total	100	1	1

[a]Mineral mix: calcium phosphate, 17.500 g; sodium chloride, 2.590 g; potassium citrate monohydrate, 7.700 g; potassium sulfate, 1.820 g; magnesium oxide, 0.840 g; potassium iodate, 0.350 mg; manganous carbonate, 0.123 g; chromium potassium sulfate, 19.250 mg; sodium selenite, 0.350 mg; ferric citrate, 0.210 g; cupric carbonate, 10.500 mg; zinc carbonate, 0.056 g (for only group II); sucrose, 4.187 g for group I (finely powdered 35.000 g); and sucrose, 4.130 g for group II (finely powdered 35.000 g).

[b]Vitamin mix: thiamine hydrochloride, 0.600 g; riboflavin, 0.600 g; pyridoxine hydrochloride, 0.700 g; nicotinic acid, 3.000 g; D-calcium pantothenate, 1.600 g; cyanocobalamin, 1.000 mg; vitamin A, 0.800 g; DL-alpha-tocopherol acetate, 0.400 g; cholecalciferol, 1.000 mg; menaquinone, 3.000 mg; folic acid, 0.200 g; D-biotin, 0.02 g, sucrose, 2.079 g (finely powdered 10.000 g).

females and two males), and group II (fed with a zinc-containing diet) contained seven rats (four females and three males).

The rats were fed similar nutrition, except zinc. The first observation was growth retardation, poor appetite, loss of hair, diarrhea, and ulcerations of the skin and mucosa, in zinc-deficient rats. The distribution of aphthous ulcer is shown in Table 2. In the present study, aphthous ulcer was observed on the alveolar mucosa with a high rate of 29.9%. Although aphthous ulcer was often seen in the study group, it was not found in the control group. These findings were first observed at 10–16 d in zinc-deficient rats. Although body weight, body length, and tail length were retarded in zinc-deficient rats, these physical properties were advanced in rats fed with a zinc-containing diet (Fig. 1).

The clinical periodontal findings and statistical comparisons are given in Table 3. The mean plaque index for group II was lower than that for group I ($p < 0.001$). A similar evaluation was also made for gingival index values (Table 3), where the mean gingival index for group II was lower than that for group I ($p < 0.001$). No significant difference, as regards pocket depth, was found between the two groups of rats ($p > 0.05$) (Table 3).

The serum zinc level of the zinc-deficient rats (group I) was lower than that of the controls (group II) ($p < 0.001$) (Table 4).

Histological findings were as followings: in comparison with those of the control group (Fig. 2A), thickening of the stratum corneum (i.e. hyperkeratosis), was found on the dorsal surface of the tongue in zinc-deficient rats (Fig. 2B). This hyperkeratinization was much more prominent in the valleys between the papillae. Additionally, pyknotic cells with condensed nuclei were observed within the keratin layer covering the tongue of zinc-deficient rats, suggesting parakeratinization (Fig. 2B). There was no difference related to the epithelial keratinization of the hard palate between the two groups (Fig. 3).

Table 2. The distribution of minor aphthous ulcers in the group I rats

Group I rats	Hard palate	Attached gingiva	Alveolar mucosa	Buccal-labial mucosa	Floor of mouth	Tongue	Total
1	1	1	3	4	0	2	11
2	1	1	4	3	0	2	11
3	1	1	3	2	1	3	11
4	1	1	4	3	1	3	13
5	2	1	3	3	0	2	11
6	1	0	2	2	1	2	8
7	1	1	4	3	0	3	12
Total	8	6	23	20	3	17	77

Values represent the number of ulcers.

Discussion

It is known that rats are more susceptible than other animals to zinc deficiency. Studies with rats therefore

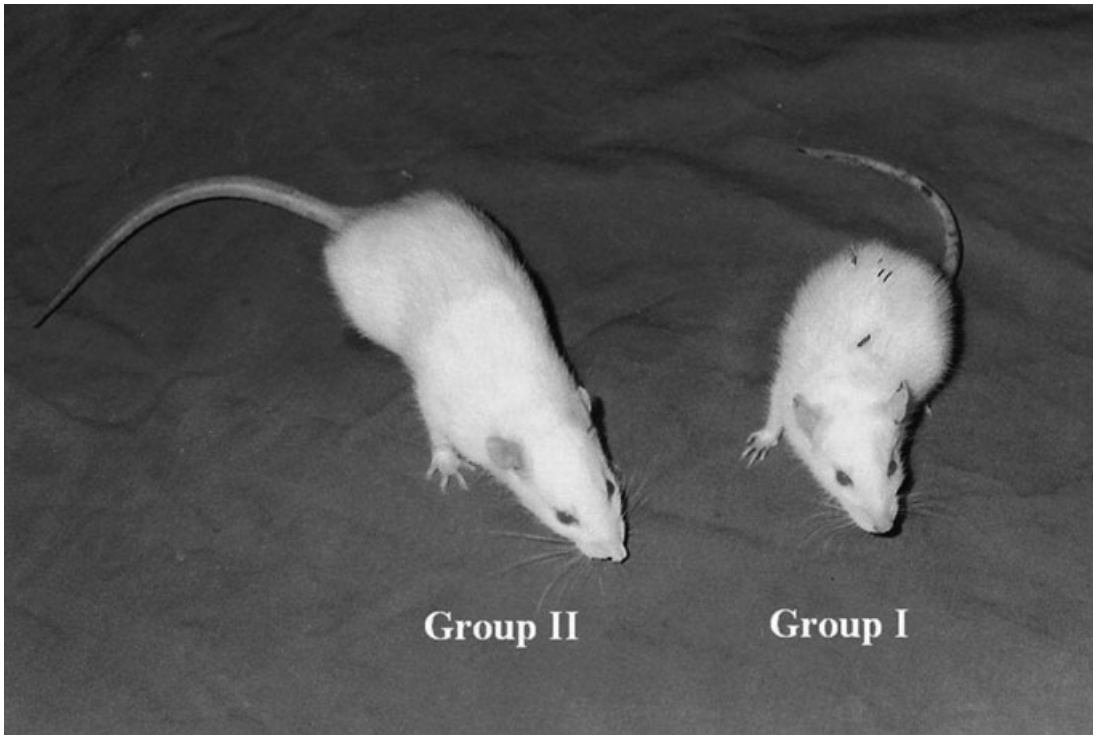

Fig. 1. Group I rats were given a zinc-deficient diet. Group II rats (controls) were given a zinc-containing diet.

Table 3. Comparison of plaque index, gingival index and pocket depth values obtained in both groups

	Plaque index			Gingival index			Pocket depth (mm)		
	n	(mean ± SD)	*p*-value	*n*	(mean ± SD)	*p*-value	*n*	(mean ± SD)	*p*-value
Group I	7	1.97 ± 0.26	< 0.001	7	2.23 ± 0.31	< 0.001	7	0.84 ± 0.24	> 0.05
Group II	7	0.90 ± 0.28		7	0.83 ± 0.25		7	0.67 ± 0.26	

Table 4. Comparison of serum zinc levels (μg/dL) of group I and group II

	n	Mean ± SD	*p*-value
Group I	7	0.84 ± 0.26	< 0.001
Group II	7	3.80 ± 0.28	

provide a useful model for investigating the effects of dietary zinc deficiency on oral tissues. We also observed oral manifestations in rats given a zinc-deficient diet.

Zinc is the second most abundant trace metal in the human body and is present in all living cells and body secretions (21). In addition, it has been reported that zinc deficiency might produce marked effects on virtually all components of the immune system (22). Our observations of growth retardation in zinc-deficient rats and growth acceleration in rats receiving supplementary zinc have confirmed that zinc is an important element for growth (2).

Although clinical features are important, zinc deficiency must be confirmed by laboratory findings (23). Zinc status has been evaluated in many studies by direct analysis of zinc concentrations in serum using atomic absorption spectrophotometry (3,24, 25). We also used this method. We identified that the serum zinc level of zinc-deficient rats was lower than that of the rats given a zinc-containing diet ($p < 0.001$).

Even though aphthous ulcers have a multifactorial etiology, recent articles suggest that patients with aphthous ulcers may have primary immune abnormalities or immune deficiency (22,25–27). For this reason, immunomodulatory drugs are used in the treatment of aphthous ulcers. In addition, nutrition is very important for the immune system. Abnormal nutrition is responsible for many diseases and aphthous ulcers, and deficiencies in iron, folic acid, zinc, and vitamins B1, B2, B6 and B12 have been detected in patents with aphthous ulcers. During the last decade, the nutrient zinc, and its immunoregulatory properties, have been a focus of considerable interest. In this study, we investigated the changes associated with zinc deficiency in the oral tissues. Aphthous ulcers or aphthous stomatitis is one of the most common oral mucosal diseases (26). Aphthous stomatitis is classified into three types, namely the minor, major and herpetiform aphthous ulcerations (26,27). The most common form of aphthous ulcers is minor aphthous ulceration, and the minor form is, respectively, followed by major and herpetiform ulceration (27). Although aphthous stomatitis can often affect buccal and labial mucosa, floor of the mouth, free gingiva, ventral surface of the tongue, soft palatal and oropharyngeal mucosa, they uncommonly involve the attached gingiva, hard palatal mucosa, vermillion border, and dorsal aspect of the tongue (26,27).

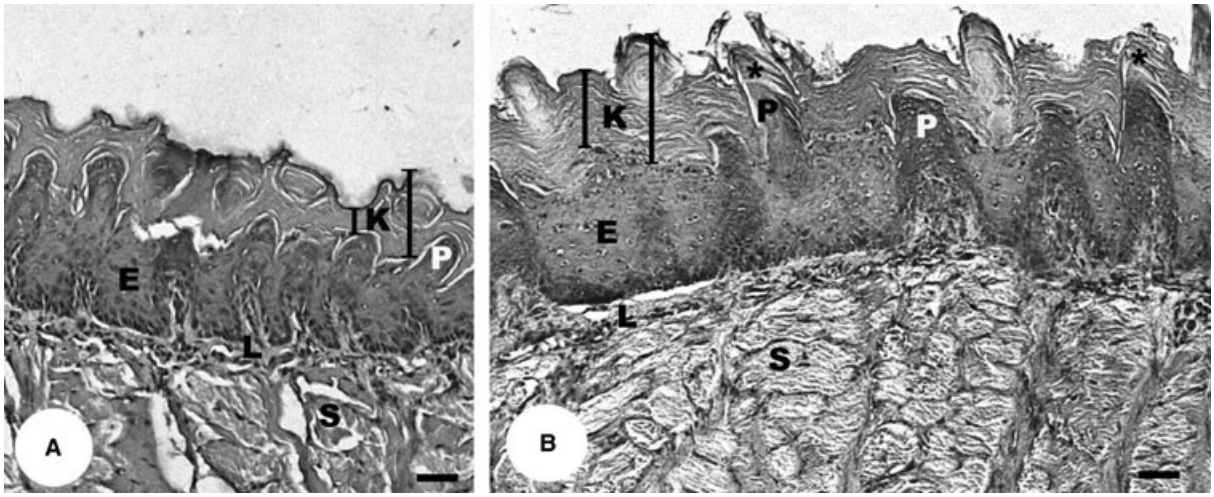

Fig. 2. (A) A light micrograph showing the dorsal surface of the tongue from control-group rats. (B) A light micrograph showing the dorsal surface of the tongue from zinc-deficient rats. E, epithelial cell layers of the tongue; K, keratin layer (or stratum corneum) of the tongue epithelium; L, lamina propria; P, papilla; S, skeletal muscle. The asterisks indicate the areas of parakeratinization. Hematoxylin and eosin stain. Bars, 100 μm.

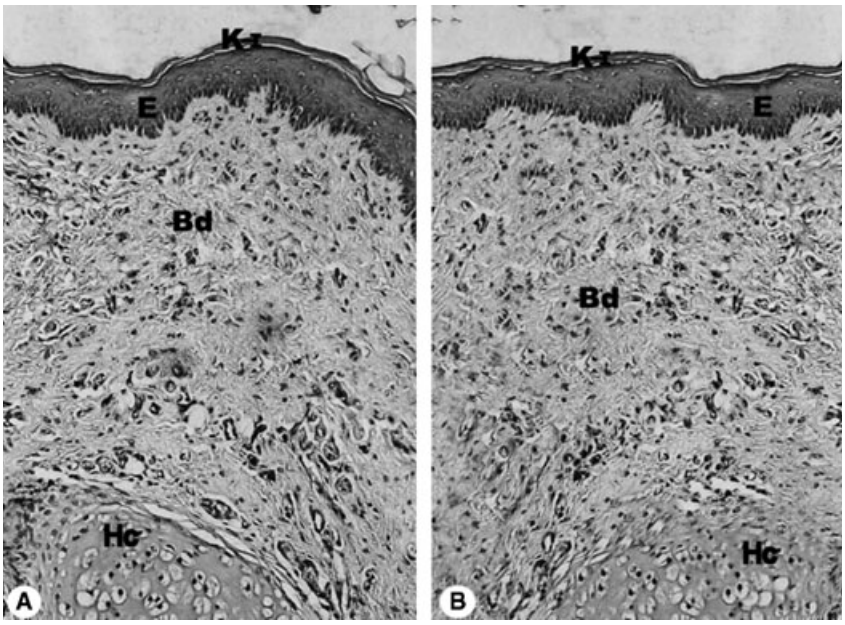

Fig. 3. (A) A light micrograph showing the hard palate from control-group rats. (B) A light micrograph showing the hard palate from zinc-deficient rats. E, epithelial cell layers of the hard palate; Hc, hyaline cartilage; K, keratin layer (or stratum corneum) of the hard palate epithelium. Hematoxylin and eosin stain. Bars, 100 μm.

It is known that microbial plaque is the main etiological factor of periodontal diseases. However, a wide range of background factors, such as age, gender, oral hygiene status, and nutrition have been identified as risk factors for the occurrence of periodontal diseases (28). In the present study, the mean plaque index for group II was lower than that for group I ($p < 0.001$).

Gingival bleeding has a positive correlation with the accumulation of plaque. As microbial plaque, which causes periodontal disease, colonizes the subgingival area, an increase should be expected in the inflammatory response of the host. Bleeding is observed in gingival tissue in infected areas (28). In our study, a statistically significant difference in gingival index values was found between group I and group II ($p < 0.001$).

One of the most commonly used clinical parameters in the diagnosis and prognosis of periodontal diseases is the periodontal pockets. In our study, periodontal pocket depth was increased. However, no significant difference of pocket depth values between the two groups was found ($p > 0.05$).

Rats and mice fed a zinc-deficient diet develop parakeratosis of normally orthokeratinized oral mucosa (29). Parakeratosis seems to be a generalized alteration in stratified squamous epithelium of zinc-deficient animals, independent of whether the epithelium is normally non-keratinized or orthokeratinized. In our study, a similar parakeratotic change occurred in the normally keratinized dorsum of the tongue. The hyperkeratinization was seen in the valleys between papillae.

Consequently, oral health was better in group II rats (those fed with a zinc-containing diet) than in group I rats (those fed with a zinc-deficient diet). Hence, zinc deficiency can be a potential risk factor for oral and periodontal diseases.

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