Original paper

Dental Materials Journal 22(1) : 21−29, 2003

Effects of Zinc Treatment in Patients with Recurrent Aphthous Stomatitis

Recep ORBAK, Yasin CICEK, Adnan TEZEL
and Yusuf DOGRU[1]

Department of Periodontology
[1]Department of Biochemistry,
Ataturk University Faculty of Dentistry, Erzurun-Turkey

Received October 18, 2002/Accepted December 25, 2002

The purpose of this study was to assess the effects of systemic zinc sulfate in the treatment of recurrent aphthous stomatitis (RAS) by atomic absorption spectrophotometry. The study was carried out on 40 patients with RAS. The first group consisted of 20 subjects with RAS who were administered zinc sulfate (220 mg) once per day for one month. In the second group, there were 20 subjects with RAS who were administered placebo (saccharose). Results showed that the levels of serum zinc before treatment were under the normal value in the 42.5% percent of the patients with RAS. Saliva ALP, serum zinc, serum albumin, and serum alkaline phosphates activity for group II were significantly lower than those for group I after treatment ($p<0.01$). After 1 month of zinc therapy the aphthae reduced and did not reappear for 3 months. The empirical use of systemic zinc sulfate supplementation in the treatment of RAS is recommend.

Key words : Recurrent aphthous ulcers, Treatment, Zinc sulfate

INTRODUCTION

Recurrent aphthous ulcers or recurrent aphthous stomatitis is one of the most common oral mucosal diseases[1−4]. Recurrent aphthous stomatitis is classified into 3 types, namely the minor, major and herpetiform aphthous ulcerations[1,5,6−9]. The most common form of RAS is minor aphthous ulceration, and the minor form is respectively followed by major and herpetiform ulceration[7].

Recurrent aphthous stomatitis is a frequently encountered problem in dentistry and for the most part presents a very troublesome clinical course. Although RAS can often affect buccal and labial mucosa, maxiller and mandibuler sulci, floor of the mouth, free gingiva, ventral surface of the tongue, soft palatal and oropharyngeal mucosa, they uncommonly involve the attached gingivae, hard palatal mucosa, vermillion border and dorsal aspect of the tongue[1,6,7].

Even though RAS has a multifactorial etiology, recent articles suggest that patients with RAS may have primary immune abnormalities or immune deficiency[2,3,5,6]. For this reason, immunomodulator drugs are used in the treatment of RAS. In addition, nutrition is very important for the immune system. Abnormal nutrition responsible for many diseases and RAS. So, deficiencies in iron, folic acid, zinc, and vitamin B1, B2, B6, B12 have been detected in patents with RAS. During the last

decade, the nutrient zinc (Zn), and its immunoregulatory properties have been the focus of considerable interest. The purpose of this study was to assess the short and long term effects of zinc sulfate in the treatment of recurrent aphthous stomatitis by atomic absorption spectrophotometer.

MATERIALS AND METHODS

Materials

A total of 40 subjects, aged between 13 and 51 years old, who were admitted to the Periodontology Department of Ataturk University Faculty of Dentistry with recurrent aphthous stomatitis complaints between June, 2000 and May, 2002 were examined. With respect to the investigation protocol, subjects were divided into two equal groups. In the first group (group Ⅰ), the individuals were administered zinc sulfate (220 mg, once per day before a meal) for one month. The second group (control: group Ⅱ) were administered placebo (saccharose, once per day before a meal) for one month. All subjects were of similar age, gender, socio-economic and socio-cultural background, and did not suffer from an oral or systemic disease apart from recurrent aphthous stomatitis.

The subjects included in the study had similar inclusion/exclusion criteria including the following:[10]

1 - A history of recurrent minor aphthous ulcers,
2 - The presence of 1 to 3 minor aphthous ulcers of less than 48 hours duration in an area of the mouth easily accessible for paste application,
3 - Not have undergone dental surgery within 2 weeks of study entry or be using orthodontic braces or an orthodontic retainer that could come into contact with the ulcer,
4 - >10 years of age,
5 - Not be pregnant or lactating,
6 - Not have any concurrent clinical condition that could pose a health risk to the subject by being involved with the study,
8 - Not have ulcers that are manifestations of a systemic disease process such as ulcerative colitis, Crohn's disease, Behcet's syndrome, or anemia,
9 - Systemic corticosteroid and immunomodulatory agents had not been used (1 month),
10 - Not taken non-stercidal anti-inflammatory agents (except occasional use for headaches),
11 - Not taken oral antihistamines (1 month),
12 - Topical medications and systemic antibiotics were not used (2 weeks).

The patients and parents were informed about the study and their consent was obtained. The investigation forms were recorded. At the first appointment the number of oral ulcers, their locations, recurrence and the degree of pain experienced were recorded. Then, the treatment recurrence intervals were recorded again. The patients were asked to take the medicines once per day for one month, and to attend

the clinic for control after they consumed the prescribed medicines.

Biochemical measurements

At the beginning of the experiment, blood and saliva samples were obtained from all the RAS subjects. Approximately 3.0 ml of unstimulated pooled saliva was collected from each subject 1 hour after the last food or drink consumption between 1 and 2 p.m. Approximately 5.0-ml peripheral blood samples were taken by venipuncture from each subject's right arm at the same time.

Blood and saliva samples were centrifuged at 5,000 rpm for 10 minutes and maintained at −20℃ until shortly before assay. Blood samples assayed for alkaline phosphates (ALP) (IU/dL), albumin (ALB) (G/dL) and zinc but saliva samples were only assayed ALP (IU/dL). While ALP and ALB were measured using an automatic analyzer (HITACHI 717 Japan), zinc was measured by atomic absorption spectrophotometry (Flame type UNICAM 929). The normal serum zinc level was accepted as 95-130μgr/dL. 220 mg zinc sulfate capsules (50 mg of elemental zinc) was administered to the patients in one group (group I), while placebo (saccharose capsules) to the other patient group (group II). Blood and saliva samples were taken again from all subjects after one month. Each patient was re-examined at every ulcer examination for 2 years.

Statistical analysis

The two groups were compared using Student's t test. In addition, the recurrence period was analysed using the Kolmogorov-Smirnov two sample test.

RESULTS

The investigation was carried out on 40 patients with recurrent aphthous stomatitis. Group I consisted of 20 patients with recurrent aphthous stomatitis (fifteen females, five males), age 26.90±10.18 years; group II contained 20 patients with recurrent aphthous stomatitis (eleven females, nine males), age 29.25±9.37 years.

The distribution of the recurrent aphthous stomatitis is given in Table 1. It was observed on the alveoler mucosa with a high a rate of 29.9%.

The levels of serum zinc before treatment were under the normal value (normal:

Table 1 The location of the minor aphthous ulcer

	Number of ulcers	%
Hard palate	3	3.9
Attached gingiva, maxillary	2	2.6
Attached gingiva, mandibular	4	5.2
Alveolar mucosa	23	29.9
Buccal and labial mucosa	20	25.9
Floor of mouth	8	10.4
Tongue	17	22.1

95 μg/dL) in the 42.5% percent of the patients with RAS. The serum zinc levels were normalized with this treatment.

According to before and after the treatment, saliva ALP, serum ALP, serum ALB and serum zinc levels of group I and group II were compared with each other (Table 2). In the first group, serum albumin (ALB) and serum alkaline phosphates activity (ALP) levels for group I before treatment was lower than those for group I after treatment, and the difference between before and after the treatment was found to be significant ($p<0.01$). In addition, in the same group, saliva ALP, serum zinc levels for group I before treatment was lower than those for group I after treatment, and the difference between before and after the treatment was found to be significant ($p<0.05$). A similar evaluation was made for group II. However, the saliva ALP, serum ALP, serum ALB and serum zinc levels there was no significant difference between before treatment and after treatment ($p>0.05$).

The recurrences of aphthous stomatitis before treatment and after treatment, and the associated statistical comparisons are given in Table 3. In the present study, the frequency of ulceration occurred at 1 week to 1 month intervals, but was most often at 1 month intervals. For before treatment, there was no significant difference between the recurrence scores of aphthous stomatitis of groups I and II ($p>0.05$). However, the recurrence scores of aphthous stomatitis for group I were lower than those for group II after treatment, and the difference between groups I and II was significant ($p<0.01$). After 1 month of zinc therapy the aphthae disappeared and did not reappear for 3 months.

Table 2 Comparison of saliva ALP (IU/dL), serum ALP (IU/dL) activity, serum ALB (G/dL) and serum zinc (μgr/dL) levels of group I and group II before and after treatment

	Group I				Group II			
		Before	After			Before	After	
	N	Mean±SD	Mean±SD	p	N	Mean±SD	Mean±SD	p
Saliva ALP	20	81.9±76.3	93.7±91.9	<0.05	20	68.8±68.3	58.2±57.8	>0.05
Serum ALP	20	173.2±66.5	182.3±63.7	<0.01	20	153.1±41.1	151.2±33.4	>0.05
Serum ALB	20	4.3±0.2	4.5±0.3	<0.01	20	4.0±0.3	4.0±0.2	>0.05
Serum Zinc	20	103.5±26.3	122.8±21.5	<0.05	20	105.6±32.2	113.5±19.9	>0.05

Table 3 The recurrences of aphthous stomatitis before treatment and after treatment in the two groups, and statistical comparisons according to the Kolmogorov-Smirnov two sample test before and after treatment

	Group I			Group II				
	1 week	1 month	3 months	1 week	1 month	3 months	X^2	P
Before treatment	9	11	–	7	12	1	0.40	>0.05
After treatment	–	2	6	5	13	2	9.66	<0.01

DISCUSSION

Minor recurrent aphthous stomatitis (RAS) is a human disease characterized by small round or oval ulcerations of the oral mucous membranes which exhibit periodic recurrences[11].

Zinc is the second most abundant trace metal in the human body and is present in all living cells and body secretions[12]. Zinc is an essential nutrition for growth in human, animals, and plants[13-16].

It was reported that zinc deficiency can have marked effects on virtually all components of the immune system[14]. In the present study, we assessed the effects of zinc sulfate in the treatment of recurrent aphthous stomatitis using atomic absorption spectrophotometery.

The clinical manifestations in severely zinc deficient subjects include bullous-pustular dermatitis, diarrhea, alopecia, mental disturbances, and intercurrent infections due to cell-mediated immune disorders: if untreated the zinc deficiency becomes fatal[17]. Growth retardation, male hypogonadism, skin changes, poor appetite, mental lethargy, abnormal dark adaptation, delayed wound healing are some manifestations of moderate zinc deficiency in human subjects[17,18]. Although clinical, biochemical, and diagnostic aspects of severe and moderate levels of zinc deficiency in humans are now well recognized, the recognition of mild marginal zinc deficiency has been difficult[17]. If zinc deficiency in the diet is continued for months or years, a mild or marginal zinc deficiency may occur. Generalized malnutrition and select nutrient deficiencies can result in a compromised immune response[14]. The influence of a nutrient on the immune response involves a complex interaction among at least three major effector mechanisms: antibody-mediated immunity, cell-mediated immunity, and phagocytosis. Although not considered a component of the immune response, mucosal and barier immunity (*i.e.* skin acting as a barrier to prevent penetration of organisms) can be affected by the nutritional status of an individual[14].

It was suggested that both blood and saliva were collected at the same time to provide consistency in the interpretation of results[19-21]. For this reason, all samples were obtained between 1-2 p.m. Mixed saliva samples and blood samples were taken at approximately 1 hour after the last food or drink. The oral cavity was rinsed three times with deionized water before collection. Protection of samples is also very important. In previous studies, the samples were maintained between $-20℃$ and $-70℃$[4,18,20,23-24]. This is changed according to assay time and sample parameters. We immediately froze the samples at $-20℃$ and maintained them at that temperature until shortly before assay.

A number of parameters have been used in the assessment of zinc concentration in serum and saliva[4,18,19,24,25], the activity of various zinc-dependent enzymes such as ALP, and the albumin concentrations in serum have been used in a number of studies[15,26,27]. All of these parameters were used in the present study.

Saliva zinc levels were used as potential indicators of zinc status[28]. Initial saliva

ALP activity was significant between the healthy and zinc sulfate groups ($p<0.05$) but there was no significant difference between the placebo and zinc sulfate groups ($p>0.05$). Consequently, saliva ALP activity of RAS patients was found to be low. However, there was no significant difference after the treatment of zinc sulfate ($p>0.05$). For this reason, it is suggested that there was no significant effect of the short term zinc treatment on saliva ALP activity.

Serum ALP activity was evaluated between the groups and within the groups. There was a significant difference between the placebo and zinc sulfate groups after the treatment. ALP activity may increase following zinc sulfate treatment. It was reported that ALP activity is increased after treatment with zinc sulfate[15,16,29]. The present results are similar to these of Baer *et al.*[26] They reported that two weeks of zinc repletion caused significant increases in total serum ALP.

Approximately 60-70% of circulating zinc is bound loosely to albumin[13]. There is a linear relation between albumin concentration and zinc concentration in the blood[30]. In the present study, serum ALB levels were used as a parameter in evaluation of serum zinc. After treatment there was a significant difference between group I and group II. Solomons[15] reported a linear relation between albumin concentration and zinc concentration in the blood. The present results and that of Solomons were similar.

Zinc status was evaluated in many studies by direct analysis of zinc concentrations in serum by atomic absorption spectrophotometry[16,25,31,32]. We also used this method. According to before treatment, there was no significant difference between group I and group II ($p>0.05$). However, there was a significant difference between group I and group II ($p<0.05$). The present results and those of Wang *et al.*[33] suggested that zinc sulfate treatment increased the serum zinc level.

In comparisons of group II before treatment and after treatment there were no significant differences for any parameters ($p>0.05$) but there was a significant difference within the groups in group I ($p<0.01$). Saliva ALP values were not significantly different between the groups before treatment and after treatment but there was a significant difference within the groups after the treatment ($p<0.05$). This suggested that saliva ALP levels can be affected very little after the zinc repletion period.

Previous studies reported that zinc can successfully be used in the treatment of RAS[31-33]. However, zinc is not taken in excess of the Recommended Dietary Allowance (RDA) because the amounts used in pharmacological dosages of 100-300 mg zn/dl may induce acute toxicity[34]. The World Health Organization recommendation for zinc is 15-100 mg/day[22,34]. However, the rate of zinc intake can be changed from one study to another.

Solomons *et al.*[35] suggested that the oral zinc intake of a patient should consist of 220 mg zinc sulfate (50 mg of elemental zinc) one times daily for 28 days, and Antoniou *et al.*[36] reported that zinc administered orally at 168 mg/day as zinc acetate (50 mg of elemental zinc) should be one times daily for 2-3 weeks. In the present study, the group I subjects were asked to take one 220 mg zinc sulfate capsule

each day for 1 month (30 days). This dosage is suitable according to the WHO recommendation.

The frequency of ulceration and its severity varies from patient to patient[37]. Recurrences usually occur at one- to four-month intervals although some patients reported a history of ulcers being present for most of the time[4,8,9]. Meiller *et al.*[38] reported that approximately one in 10 persons is susceptible to monthly attacks, whereas most susceptible persons have three to four episodes of new lesions per year. In addition, Woo *et al.*[7] reported that greater than 50 percent of patients had an episode every one to three months, while 30 percent of patients had continuous recurrences without ulcer-free periods. In the present study, improvement consisted of 80% to 100% reduction of the frequency of episodes. After 1 month of zinc therapy, the aphthae ulceration disappeared in 1 week intervals. In addition, improvement consisted of 81.8% reduction the frequency of episodes at 1 month intervals. No therapeutic effect was seen with the use placebo. At baseline, recurrences of aphthous did not exhibit any statistically significant differences between group I and group II ($p>0.05$), but after the treatment there was a significant differences between the groups ($p<0.01$).

CONCLUSIONS

Of the 40 patients with RAS, 17 (42,5%) were found to have serum zinc levels less than normal. These results suggest that in some patients there is an association between zinc deficiency and RAS. After 1 month of zinc therapy (220 mg zinc sulfate orally once per day for one month) the aphthae were reduced and did not reappear for 3 months. Improvement consisted of 80% to 100% reduction in the frequency of episodes. No side effect was seen with use systemic zinc sulfate. The serum zinc levels were also normalized with treatment. The empirical use of systemic zinc sulfate supplementation in the treatment of RAS is recommend.

REFERENCES

1) Ship, J. A.: Recurrent aphthous stomatitis: an update, *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* **81** : 141-147, 1996.
2) Ylikontiola. L., Sorsa, T., Häyrinen-Immonen, R., Salo, T.: Doxymycine-cyanoacrylate treatment of recurrent aphthous ulcers, *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* **83** : 329-333, 1997.
3) Brice, S. L.: Clinical evaluation of the use of low-intensity ultrasound in the treatment of recurrent aphthous stomatitis, *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* **83** : 14-20, 1997.
4) Wu-Wang, C. Y., Patel, M., Feng, J., Milles, M., Wang, S. L.: Decreased levels of salivary prostoglandin E2 and epidermal growth factor in recurrent aphthous stomatitis, *Archs Oral Biol* **40**(12) : 1093-1098, 1995.
5) Cooke, B. E. D.: Recurrent oral ulceration, *Br J Dermatol* **81** : 159-161, 1969.
6) Neville, B. W., Damm, D. D., Allen, C. M., Bouquot, J. E.: Oral and maxillofacial pathology, 5 th ed., W. B. Saunders Co., Philadelphia, 1995, pp.236-241.
7) Woo, S. B., Sonis, T. S.: Recurrent aphthous ulcers: a review of diagnosis and treatment,

JADA **127** : 1202-1213, 1996.
8) Rennie, J. S., Reade, P. C., Scully, C.: Recurrent aphthous stomatitis, *Br Dent J* **159** : 361-367, 1985.
9) Scully, C., Porter, S. R.: Recurrent aphthous stomatitis: current concepts of etiology, pathogenesis and management, *J Oral Pathol Med;* **18** : 21-27, 1989.
10) Khandwala, A., Inwegen, R. G. V., Alfano, M. C.: 5% Amlexanox oral paste, a new treatment for recurrent minor aphthous ulcers. I: Clinical demonstration of acceleration of healing and resolution of pain, *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* **83** : 222-230, 1997.
11) Eversole, L. R., Shopper, T. P., Chambers, D. W.: Effects of suspected foodstuff challenging agents in the etiology of recurrent aphthous stomatitis, *Oral Surg Oral Med Oral Pathol* **54** : 33-38, 1982.
12) Lansdown, A. B. G.: Zinc in the healing wound, *Lancet* **347** : 706-707, 1996.
13) Fickel, J. J., Freeland-Graves, J. H,, Roby, M. J.: Zinc tolerance tests in zinc deficient and zinc supplemented diets, *Am J Clin Nutr;* **43** : 47-58, 1986.
14) Keen, C. L.: Zinc deficiency and immune function, *Annu Rev Nutr* **10** : 415-431, 1990.
15) Solomons, N. W.: On the assessment of zinc and copper nutriture in man, *Am J Clin Nutr* **32** : 856-871, 1979.
16) Bates, J., McClain, C. J.: The effect of severe zinc deficiency on serum levels of albumin, transferrin, and prealbumin man, *Am J Clin Nutr* **34** : 1655-1660, 1981.
17) Prasad, A. S.: Discovery of human zinc deficiency and studies in an experimental human model, *Am J Clin Nutr* **53** : 403-412, 1991.
18) Bales, C. W., Freeland-Graves, J. H., Askey, S., Behmardi, F., Robocik, R. S., Fickel, J. S., Greenlee, P.: Zinc, magnesium, copper and protein concentrations in human saliva: age-and sex-related differences, *Am J Clin Nutr* **51** : 462-469, 1990.
19) Freeland-Graves, J. H., Hendrickson, P. J., Ebangit, M. L., Snowden, J. Y.: Salivary zinc as an index of zinc status in women fed a low-zinc diet, *Am J Clin Nutr* **34** : 312-321, 1981.
20) Meurman, J. H., Collin, H. L., Niskanen, L., Toyry, J., Alakuijala, P., Keinanen, S., Vusitupa, M.: Saliva in non-insulin-dependent diabetic patients and control subjects, *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* **86** : 69-76, 1998.
21) Wright, A. L., King, J. C., Baer, M. T., Citron, L. J.: Experimental zinc depletion and altered taste perception for NaCl in young adult males, *Am J Clin Nutr* **34** : 848-852, 1981.
22) Maragou, P., Ivanyi, L.: Serum zinc levels in patients with burning mouth syndrome, *Oral Surg Oral Med Oral Pathol* **71** : 447-450, 1991.
23) Toro, R. D., Capotorti, M. G., Gialanella, G., Giudice, M. M., Moro, R., Perrone, L.: Zinc and copper status allergic children, *Acta Pediatr Scand* **76** : 612-617, 1987.
24) Etzel, K. R., Hempel, J. D., Keepsel, R. R.: Identification of zinc proteins in rat parotid saliva, *Archs Oral Biol* **42**(2) : 173-179, 1997.
25) Prasad, A. S.: Laboratory diagnosis of zinc deficiency, *J Am Col Nut* **4** : 591-598, 1985.
26) Baer, M. T., King, J. C., Tamura, T., Margen, S., Bradfield, R. B., Daugherty, N. A.: Nitrogen utilization, enzyme activity glucose intolerance and leukocyte chemotaxis in human experimental zinc depletion, *Am J Clin Nutr 1985;* **44** : 1220-1235.
27) Taylor, C. M., Bacon, J. R., Aggett, P. J., Bremner, I.: Homeostatic regulation of zinc absorption and endogenous losses in zinc-deprived men, *Am J Clin Nutr* **53** : 755-763, 1991.
28) Greger, J. L., Sickle, V. S.: Saliva zinc levels potential indicators of zinc status, *Am J Clin Nutr* **32** : 1859-1866, 1979.
29) Wray, D.: A double-blind trial of systemic zinc sulfate in recurrent aphthous stomatitis, *Oral Surg Oral Med Oral Pathol* **53**(5) : 469-472, 1982.
30) Ivanyi, L., Maragou, P.: Serum zinc levels in patients with burning mouth syndrome, *Oral Surg Oral Med Oral Pathol* **71** : 447-450, 1991.
31) Endre, L.: Recurrent aphthous ulceration with zinc deficiency and cellular immune deficiency, *Oral Surg Oral Med Oral Pathol* **72** : 559-561, 1991.
32) Merchant, H. W., Gangarosa, L. P., Glassman, A. B., Sobel, R. E.: Zinc sulfate

supplementation for treatment of recurring oral ulcers, *South Med J* **70**(5) : 559-561, 1977.
33) Wang, S. W., Li, H. K., He, J. S., Yin, T. A.: Le zinc, oligoelement et l'aphtose Le dosage du zinc plasmatique et le traitment de l'aphtose par le zinc, *Rev Stomatol Chir Maxillofac* **87**(5) : 339-343, 1986.
34) Fosmire, G. J.: Zinc toxicity, *Am J Clin Nutr* **51** : 225-227, 1990.
35) Solomons, N. W., Guerrero, A. M., Torun, B., Johnson, P., Milne, D. B.: Studies on the feedback regulation of zinc absorption in humans, *Fed Proc* (*Abstract*) **42** : 823, 1983.
36) Antoniou, C. D., Shalhoub, R. J., Elliot, S.: Zinc tolerance tests in chronic uremia, *Clin Nephrol* **16** : 181-187, 1981.
37) Addy, M., Tapper-Jones, L., Seal, M.: Trial of astringent and antibacterial mouthwashes in the management of recurent aphthous ulceration, *Brit Dent J* **136** : 452-455, 1974.
38) Meiller, T. F., Kutcher, M. J., Overholser, C. D., Niehaus, C., Depaola, L. G., Siegel, M. A.: Effect of an antimicrobial mouthrinse on recurrent aphthous ulcerations, *Oral Surg Oral Med Oral Pathol* **72** : 425-429, 1991.