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Do Heavy Metals Accumulated in Saliva Involve in the Etiopathogenesis of Recurrent Aphthous Stomatitis?

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Abstract

Recurrent aphthous stomatitis (RAS) *is one of the most common diseases* of oral mucosa characterized by recurrent painful ulcers. Although many factors have been implicated in its etiology, they are not fully identified. To investigate the involvement of heavy metals accumulated in saliva in the etiopathogenesis of RAS. This is a prospective, comparative, and controlled clinical study investigating the relationship between heavy metal exposure and RAS. The study consisted of 75 patients with idiopathic RAS who presented to our clinic with recurrent oral wound complaints and 74 healthy volunteers. All subjects were interviewed regarding age, acute or chronic diseases, dietary habits, and possible chemical exposure. Lead (Pb), cadmium (Cd), mercury (Hg), copper (Cu), and manganese (Mn) was measured in saliva by inductively coupled plasma–mass spectrometer (ICP–MS). Significance was considered at $p < 0.05$. Salivary levels of Pb (15.2 ± 1.1 vs. 7.6 ± 9.9 μg/l; $p < 0.003$), Hg (0.50 ± 0.60 vs. 0.13 ± 0.30 μg/l; $p < 0.001$; $p < 0.001$), Cd (0.11 ± 0.14 vs. 0.06 ± 0.13 μg/l; $p < 0.021$), and Cu (34.9 ± 22.5 vs. 21.6 ± 21.9 μg/l; $p < 0.001$) were significantly higher in the patient group than that in the control group. There was no significant difference between Mn levels (57.9 ± 41.6 μg/l). Higher heavy metal content of saliva in the patients with RAS may induce apoptosis and ulcer in oral mucosa cells through triggering release of reactive oxygen species resulting from oxidative stress resulting DNA damage.

Keywords Recurrent Aphthous stomatitis · Saliva · Heavy metals · Oxidative stress

Introduction

Recurrent aphthous stomatitis (RAS) *is one of the most common diseases* of oral mucosa with recurrent painful ulcers. It is a multifactorial disease with predisposing factors, of which etiology is not clear yet. Studies have suggested involvement of genetics, malnutrition, hematological deficiencies, immune deficiency conditions, microbial factors, trauma, stress, endocrinological disorders and drugs [1–5]. The lesions are characterized by a single or multiple painful ulcers of round or oval shape, with a gray or yellow background, surrounded by a sharply delimited erythematous halo, and usually occur in childhood or adulthood for the first time [6]. Three main types include minor, major, and herpetiform aphthae, which differ in size, number, duration, location of ulcerations, and potential for scarring.

The term heavy metal is used for metals with a density greater than 5 g/cm^3 [7, 8]. In medicine, the term heavy metal is used for metals which are toxic to the organism regardless of the atomic weight. Although there are more than 60 elements considered heavy metals, the most well-known and most common ones are manganese (Mn), mercury (Hg), lead (Pb), cobalt (Co), cadmium (Cd), copper (Cu), chromium (Cr), iron (Fe), nickel (Ni), arsenic (As), zinc (Zn), and silver (Ag) [7, 9, 10]. Pb, Cd, and Hg are potentially toxic heavy metals at low concentrations [11]. Some heavy metals such as Fe, Mn, Cu, Se, Zn, and Ni are required at low concentration for maintaining good health but they become toxic when taken in larger amounts [12].

As a result of the rapid increase in industrialization and urbanization in the last century, pollution of biological

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environments and foodstuffs has increased. Pathways of exposure to heavy metals include consumption through contaminated foods, inhalation, and dermal contact [13, 14]. They cannot be discarded via normal excretion routes (kidney, intestine, liver, skin, lung). Therefore, a large proportion of these non-disposable harmful heavy metals accumulate in the body and exert toxic effects on tissues.

The pathway of exposure affects the consequences and emergences of toxic effects depending on tissues [15, 16]. Toxic effects initiate with increment of oxidative stress, release of inflammatory cytokines, binding to protein groups, disruption of the function of enzyme systems, inducing DNA damage, and apoptosis [17–20], mechanisms by which heavy metals inducing RAS may result inflammation and ulcer on the oral mucosa [13]. Heavy metals accumulated in saliva may induce apoptosis and ulcer in oral mucosa by aggravated oxidative stress. This prospective, controlled clinical trial was conducted to investigate the relationship between RAS and salivary heavy metals.

Materials and Methods

Patients and Study Design

The study was conducted with the participation of University Hospital, Departments of Dermatology and Venereal Diseases and Medical Biochemistry. The study was approved by the ethics committee of University (approval date 09.06.2017, decision no. 2017/0035). All patients and healthy subjects in the control group were informed about the study and written consent was obtained.

Seventy-five patients with RAS and 74 controls were included in our study prospectively between June 2017 and April 2018. All subjects were interviewed regarding age, acute or chronic diseases, dietary habits, and possible chemical exposure. All patients with oral aphthous ulcerations that recur more than three times per year were included into the RAS group after anamnesis, physical examination, and reviewing the laboratory results.

Exclusion criteria included (1) patients with a history of systemic disease in particular Behcet's syndrome, systemic lupus erythematosus, or any other diseases with oral mucosal involvement, (2) those with a history of antibiotic, anti-inflammatory drug, immunomodulatory agents, and vitamin or antioxidant supplements usage during the 4 weeks prior to the study, (3) patients with metal restorations, tobacco consumers whose salivary biochemistry were affected, and (4) patients on a special diet.

Measurements of Metal Concentrations

Two-milliliter saliva sample was collected from each individual using the spitting method and the tubes were kept in a freezer at − 20 °C. After all samples were collected, dissolution was performed by adding acid solution (8 ml of HNO_3 + 2 ml of H_2O_2). Decomposition was performed in the system which was set to 220 °C T1 temperature for 20 min and 220 °C T2 temperature for 15 min. After adding 30 ml of distilled, samples were filtered for mineral assays (Mn, Hg, Pb, Cd, and Cu) by ICP–MS.

Table 1 Detailed characteristics of patients

Parameter	Data
Marital status	47 married, 28 single
Place of residence	61 city, 14 rural
Family history	Positive 35 (46.6%) Negative 40 (53.4%)
Mean duration of disease	> 7 years 25 (16.8%) < 7 years 50 (66.7%)
Frequency of attacks	< 10 attacks per year 14 (18.7%) 10–20 attacks per year 15 (20%) 20–30 attacks per year 23 (30.7%) > 30 attacks per year 23 (30.7%)
Active ulcer during examination	Positive 66 (88%) Negative 9 (12%)

Statistical Analysis

Statistical Package for Social Sciences (SPSS) 17.0 program was used for data analysis. The distribution of the data was checked by the Shapiro–Wilk test. Student's *t* test was used to compare the normally distributed quantitative parameters. One-way ANOVA test and post hoc Tukey's test were used to compare more than two groups. Chi-square test was used to compare categorical data. Data were expressed as mean ± standard deviation and $p < 0.05$ was considered statistically significant.

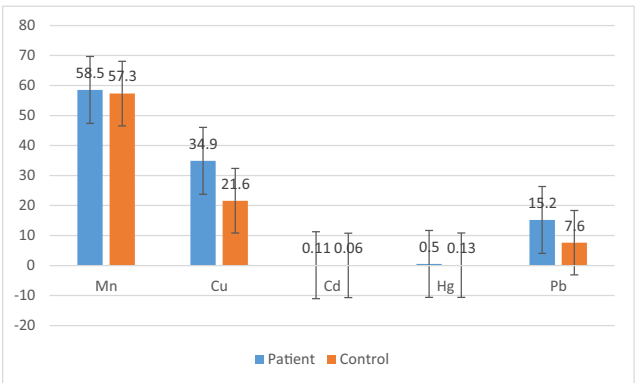

Fig. 1 Salivary heavy metals in patients and controls

Table 2 Comparison of salivary heavy metal levels in patient and control groups

Element (μg/l)	Patient	Control	*p*
Hg	0.50 ± 0.60	0.13 ± 0.30	*0.001*
Cu	34.9 ± 22.5	21.6 ± 21.9	*0.001*
Pb	15.2 ± 1.1	7.6 ± 9.9	*0.003*
Cd	0.11 ± 0.14	0.06 ± 0.13	*0.021*
Mn	58.5 ± 39.2	57.3 ± 44.1	0.890

Italic entries was used for Student's t test

Results

Demographic Findings and Disease Characteristics

This prospective study included 75 RAS patients (34 male and 41 female) with a mean age of 31.0 ± 10.7 years and 74 controls (44 male and 30 female) with a mean age 31.9 ± 12.7 years. There were not any significant differences in age or gender between the groups. Mean duration of disease, frequency of attacks, the presence of ulcers during the examination, and the other detailed characteristics of the patients are shown in Table 1.

Saliva Heavy Metal Levels

Salivary levels of Pb (15.2 ± 1.1 vs. 7.6 ± 9.9 μg/l; $p < 0.003$), Hg (0.50 ± 0.60 vs. 0.13 ± 0.30 μg/l; $p < 0.001$; $p < 0.001$), Cd (0.11 ± 0.14 vs. 0.06 ± 0.13 μg/l; $p < 0.021$), and Cu (34.9 ± 22.5 vs. 21.6 ± 21.9 μg/l; $p < 0.001$) were significantly higher in the patient group than that in the control group (Fig. 1, Table 2). There was no significant difference between Mn levels (57.9 ± 41.6 μg/l).

The relationship between disease duration, number of attacks, presence of active ulcer, family history, and place of residence and heavy metal ratios are shown in Table 3. Disease duration and presence of active ulcer caused variation in salivary Hg, whereas place of residence caused variation in both Cd and Pb.

Discussion

The relationship between RAS and heavy metals accumulation in saliva was investigated in this study. While Pb, Cd, Hg, and Cu levels were significantly higher in the patient group than that in the control group, no significant difference was observed in the Mn levels between the two groups. Since this study is the first study examining the relationship between heavy metals in saliva and RAS, there is no direct comparison of our results in the literature.

Due to the increased pollution of biological environments and foodstuff, heavy metal exposure has become a more common problem today. In a greenhouse experiment study conducted by Topçuoğlu et al., they found that the amount of Pb and Cd content exceeding the limit given for human health in the plant by applying mud increased salt and mineral content on tomato [21]. In our study, it was observed that Cd and Pb levels of the urban patients were significantly higher than those living in the village ($p = 0.04$, $p = 0.03$). Similarly, Gonzales–Reimers et al. reported that people living in urban area have higher Cd levels than populations living in rural areas [22].

Pathways of exposure to heavy metals are considered intake, inhalation, and dermal contact; in particular, most of the daily exposure to heavy metals comes from the diet [13, 14]. Currently, heavy metal exposure is experienced especially as a

Table 3 Comparison of various properties and heavy metal ratios of patients

Category	Criterion	Mn	Cu	Cd	Hg	Pb
Family story	Positive	63.4 ± 40.1	34.2 ± 24.0	0.13 ± 0.10	0.42 ± 0.50	15.2 ± 10.4
	Negative	53.8 ± 38.4	35.4 ± 21.5	0.10 ± 0.10	0.57 ± 0.60	15.2 ± 12.0
Disease duration	< 7 years	58.5 ± 39.3	33.4 ± 20.2	0.12 ± 0.10	0.60 ± 0.60*	15.2 ± 12.2
	> 7 years	58.4 ± 40.0	38.2 ± 27.4	0.10 ± 0.10	0.20 ± 0.30*	15.1 ± 7.5
Number of attacks	< 10	58.1 ± 39.8	27.2 ± 22.0	0.18 ± 0.16	0.75 ± 0.70	17.5 ± 10.1
	10–20	45.3 ± 42.9	31.2 ± 16.9	0.04 ± 0.10	0.50 ± 0.60	23.8 ± 9.2
	20–30	59.3 ± 37.4	33.2 ± 17.8	0.13 ± 0.16	0.60 ± 0.70	18.0 ± 8.9
	> 30	65.5 ± 38.9	43.3 ± 28.5	0.11 ± 0.12	0.30 ± 0.40	15.3 ± 11.4
Active ulcer	Positive	56.5 ± 36.2	35.3 ± 22.0	0.10 ± 0.14	0.40 ± 0.50*	15.2 ± 11.5
	Negative	75.7 ± 60.8	30.4 ± 29.5	0.18 ± 0.16	1.30 ± 0.2*	14.9 ± 8.5
Place of residence	Rural	48.8 ± 32.8	27.0 ± 13.1	0.05 ± 0.06*	0.50 ± 0.70	8.8 ± 7.3*
	City	60.8 ± 40.5	36.8 ± 24.0	0.13 ± 0.16*	0.50 ± 0.60	16.9 ± 11.4*

*Statistically significant ($p < 0.05$)

result of the increase in consumption of ready-made foods coated with plastic materials or in tin cans. It was found that the amount of Pb, Sn, Cd, and Fe metals increased in foodstuffs with high acid content especially in tin cans compared with those not sold in tin cans [23, 24]. Heavy metals in particular Pb, Cd, and Hg cause toxicity in living cells by following oxidative stress [13]. They may induce DNA damage and apoptosis in living cells due to increased reactive oxygen species, lipid peroxidation, deterioration of enzyme functions, and production of inflammatory cytokines [17–20]. RAS is an inflammatory disease and TNF-α is thought to play a dominant role in the pathogenesis. Also, proinflammatory cytokine levels such as interleukin-1, interleukin-6, and interleukin-8 are increased in ulcerated lesions of RAS and interleukin-10, an anti-inflammatory cytokine, is decreased [25]. Triggering factors activate T-lymphocytes and leukocyte chemotaxis so proinflammatory cytokines may increase against the oral mucosa [26]. Reactive oxygen species (ROS), lipid peroxidation, and impaired antioxidant defense system are thought to be the some triggering factors for RAS [27–30]. Babae et al. reported that the attacks of ROS on the oral mucosa can damage the normal cell functions and integrity of cell structures which may result many pathophysiological conditions such as inflammation and ulcer [27]. Zhang et al. showed high concentration of ROS in lesion area and impaired enzymatic antioxidant defense system in RAS patients [28]. Saral et al. reported that non-enzymatic antioxidant defense system was impaired and lipid peroxidation was increased in RAS patients [29]. Consequently heavy metals accumulated in saliva may induce apoptosis and ulcer in oral mucosa by increment of oxidative stress, and inflammatory cytokines, deterioration of enzyme functions, and DNA damage.

In particular, studies on the association of heavy metal exposure and diseases with unknown etiology such as infertility, ectopic pregnancy, autism, multiple sclerosis, Alzheimer's disease, systemic sclerosis, and nasal polyps have been focus of interest recently [17, 31–33]. Our study showed that RAS patients had higher salivary heavy metal content.

Limitation

There are some limitations of our study, the most important of which is the fact that heavy metal levels are not simultaneously measured in blood and saliva and then not compared.

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Compliance with Ethical Standards

The study was approved by the ethics committee of University (approval date 09.06.2017, decision no 2017/0035). All patients and healthy subjects in the control group were informed about the study and written consent was obtained.

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