Salivary organic acids in metal allergy suspected patients and non-metal allergy patients

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Purpose: The purpose of this study is to determine the characteristics of whole saliva in metal allergy suspected patients.
Materials and Methods: The participants included 25 metal allergy suspected patients and 31 non-metal allergy patients. Resting and stimulated saliva were collected from the participants and subjected to measurement of flow rate, buffer capacity, and counts of *mutans streptococci* and *lactobacilli*. Moreover, the concentration of citric, pyruvic, malic, succinic, lactic, formic, acetic, propionic and carbonic acids in the saliva was measured by high-performance liquid chromatography and assessed for differences between the metal allergy and control groups.
Results: The metal allergy group has a significantly lower flow rate in resting saliva than control group. In stimulated saliva, no significant difference was observed between the two groups. With regard to buffer capacity and microorganism counts, no significant difference was observed between the two groups. With regard to the concentration of organic acids, the concentration of pyruvic and lactic acids in resting saliva was significantly lower in the metal allergy group than in the control group. In stimulated saliva, we found no significant difference between the two groups.
Conclusion: The flow rate of resting saliva was lower in the metal allergy group than in the control group. Different concentrations of pyruvic and lactic acids were found in the resting whole saliva between the two groups. **(Int Chin J Dent 2004; 4: 107-113.)**

Clinical Significance: These findings indicate the existence of differences in the salivary characteristics between the two groups.
Key Words: flow rate, high-performance liquid chromatography, organic acid, metal allergy, whole saliva.

Introduction

The whole saliva contains various substances such as plaque, gingival crevice fluid, exfoliative gingival epithelium cells, protein, microorganisms and organic acids.[1] The concentration of these substances has been shown to change according to various factors.[2-6] The correlation between the hormone concentration in saliva and serum has also been reported.[7] Consequently, whole saliva, which can be collected by easy noninvasive methods, is attracting attention as a biochemical specimen.[8,9]

To date, a considerable number of studies have assessed the relationship between saliva and both dental caries[10,11] and restorative dental materials.[12-14] It is also a well-known fact that caries-inducing bacteria such as *mutans streptococci* and *lactobacilli* are abundant in the oral cavity where the flow rate of saliva is low and that individuals with low buffer capacity of saliva are particularly susceptible to caries.[15,16] It has been reported that lactic acid, citric acid, malic acid and phosphoric acid cause erosion of the enamel and dentin layers.[17,18] Moreover, it has been demonstrated in both in vitro and in vivo studies that most dental metals are corroded by saliva,[13,14,19] as well as by organic acids[20-23] such as lactic acid and formic acid.

It has also been surmised that metal ions released from metallic restorations cause dental metal allergy, which is the subject of increasing interest in clinical dentistry.[24-28] We suspect that the nature of whole saliva is one of

the factors influencing the separation of metal ions in the oral cavity, because whole saliva is constantly in contact with metallic appliances. However, although there have been reports on the concentration of lactic acid, citric acid, pyruvic acid, malic acid and succinic acid in whole saliva,[29-32] little is known about the concentration of other organic acids such as formic and oxalic acids that may cause intraoral corrosion of dental metal appliances.[23,33] Moreover, despite awareness of the possible influence of salivary characteristics on the detachment of metal ions as described above, limited information is available on the salivary characteristics of metal allergy patients.

The purpose of the present study is to determine the characteristics of whole saliva in metal allergy patients by comparing the flow rate, buffer capacity, microorganism counts and concentration of organic acids in metal allergy suspected patients and non-metal allergy patients.

Materials and Methods

Participants

The participants included 25 metal allergy suspected patients (10 males, 15 females; mean age and standard deviation of 55.5±12.1 years) and 31 non-metal allergy patients (10 males, 21 females; mean age and standard deviation of 53.3±13.8 years). Patients suspected of having metal allergies (hereafter referred to as the metal allergy group) are those who fulfill the following three conditions: 1) presence of leukoplakia or lichen planus on the oral mucosa or presence of blisters or eczema on the skin; 2) referral to the Nagasaki University School of Dentistry Hospital by a dermatologist, etc. who was unable to treat the condition and therefore suspected a dental metal allergy; and 3) correspondence of metal elements contained in the intraoral metallic restoration with those that showed a positive reaction to a seven-day patch test conducted according to International Contact Dermatitis Research Group standards.[34] The participants in the non-metal allergy group (referred to hereafter as the control group) were patients visiting the dental outpatient clinic who showed no symptoms of metal allergy in the oral mucosa or skin and who had no previous history of food or drug allergies. In the metal allergy group, 20 participants (69 %) were taking drugs such as anti-allergic agents, immunosuppressant agents and antihypertensive agents. These drugs have been shown to be capable of inducing dry mouth as a side effect.[35,36]

The Nagasaki University School of Dentistry Ethics Committee approved this study. All participants provided informed consent on the basis of prior explanations, and the rights of all participants were strictly observed.

Collection of saliva

Resting whole saliva and stimulated whole saliva were collected from the participants. Collection was conducted under available light, with the participant in a sitting position, after verbally confirming that at least two hours had passed since the participant ingested food or brushed his/her teeth.[1]

Resting whole saliva was absorbed on cotton rolls. One roll each was inserted on the lingual side of the right and left mandibular first molars and in the sublingual fold and allowed to absorb saliva for five minutes. After five minutes, the three cotton rolls were removed, and another roll was used to swab remaining saliva from the floor of the oral cavity and the buccal mucosa. Stimulated whole saliva, meanwhile, was collected by expectoration.[37] The participants were asked to chew on a 1.0 g paraffin pellet and to deliver the saliva collecting in the oral cavity into a measuring cylinder. Collection was conducted for a period of five minutes. The four cotton rolls used to absorb resting whole saliva and the stimulated whole saliva were centrifuged for 15

minutes at 3,000 rpm. The resting and stimulated whole saliva specimens thus obtained were measured for volume, and flow rate was calculated. The buffer capacity of stimulated saliva was determined using a buffer strip (Dentobuff strip, Orion Diagnostica, Espoo, Finland). *Mutans streptococci* (SM) and *lactobacilli* (LB) counts were determined using a plastic strip (Dentocult SM, Orion Diagnostica) and a dip-slide (Dentocult LB, Orion Diagnostica).[16] The resting and stimulated whole saliva specimens were preserved at -80˚C until analysis.

High performance liquid chromatography analysis

The frozen specimens were thawed at 37˚C, two-fold diluted in distilled water, and passed through a 0.45 μm membrane filter. Each 20 μL specimen was then subjected to analysis at 37˚C for 50 minutes per specimen using a high-performance liquid chromatography system (HPLC) (LC-10vp, Shimadzu Corporation, Kyoto, Japan). The volume of each salivary acid was measured by the absolute calibration curve method. The conditions of analysis are shown in Table 1. The acids that can be analyzed using this system are citric acid, pyruvic acid, malic acid, succinic acid, lactic acid, formic acid, acetic acid, propionic acid and carbonic acid.[38]

Table 1. Analytical conditions of high-performance liquid chromatography (HPLC).

Separation conditions
Column: Shim-pack SCR-102H (8 mm I.D. x 300 mm length) tandem connection with guard column
Mobile phase: 5 mmol/L *p*-toluene sulfonic acid Flow rate: 0.8 mL/minute Temperature: 40˚C
Detection conditions
Reagent: 20 mmol/L Bisiminotrismethane including 5 mmol/L *p*-toluene sulfonic acid and 100 mmol/L EDTA
Flow Rate: 0.8 mL/minute Detector: conductivity detector (CCD-6A, Shimadzu Corporation)
Polarity: + Response: SLOW Temperature: 43˚C Scale: $1x2^4$ μS/cm*

*S=electric current (A) / electric potential difference (V)

Statistical analysis

The data thus obtained were subjected to analysis using categorized data[39] for buffer capacity and counts of SM and LB, by the Mann-Whitney U test[40] for the concentration of acids, and the results were compared between the metal allergy group and the control group. P-values lower than 0.05 were regarded as statistically significant.

Results

Salivary flow rate

The mean flow rate and standard deviation in resting whole saliva were 0.23±0.30 mL/minute and 0.38±0.36 mL/minute in the metal allergy group and control group, respectively, revealing a significantly lower flow rate in the former ($p<0.05$). In stimulated whole saliva, the mean flow rate was 1.23±0.83 mL/minute and 1.45±0.93 mL/minute in the metal allergy group and control group, respectively, revealing no significant difference between the two groups ($p=0.79$).

The mean flow rate was also compared between participants under medication and those not under medication in metal allergy group. In resting whole saliva, the mean flow rate was 0.25±0.27 mL/minute and 0.34±0.24 mL/minute in the former and latter, respectively, revealing no significant difference between the two groups ($p=0.06$). In stimulated whole saliva, the mean flow rate was 1.22±0.77 mL/minute and 1.40±0.61 mL/minute in the former and latter, respectively, also revealing no significant difference between the two groups ($p=0.07$).

Salivary buffering capacity and microorganism counts

Salivary buffering capacity was assessed at three different levels ($pH\geq6.0$, $4.5\leq pH\leq5.5$, $pH\leq4.0$). The

proportion of each level in the metal allergy and control groups is shown in Fig. 1. No significant differences were observed between the two groups (p=0.50). *Mutans streptococci* (SM) and *lactobacilli* (LB) counts are also shown in Fig. 2. The counts tended to be higher in the metal allergy group than in the control group, but the difference was not statistically significant (SM: p=0.10; LB: p=0.31).

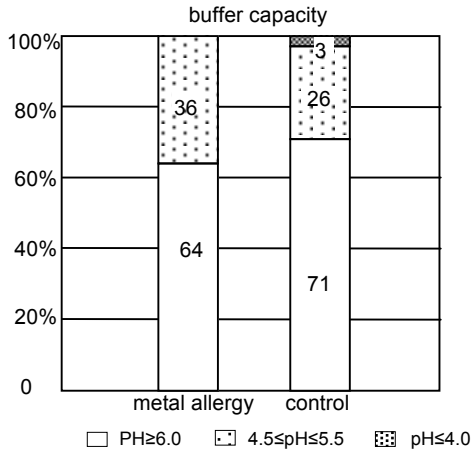

Fig. 1. Buffer capacity of stimulated saliva.

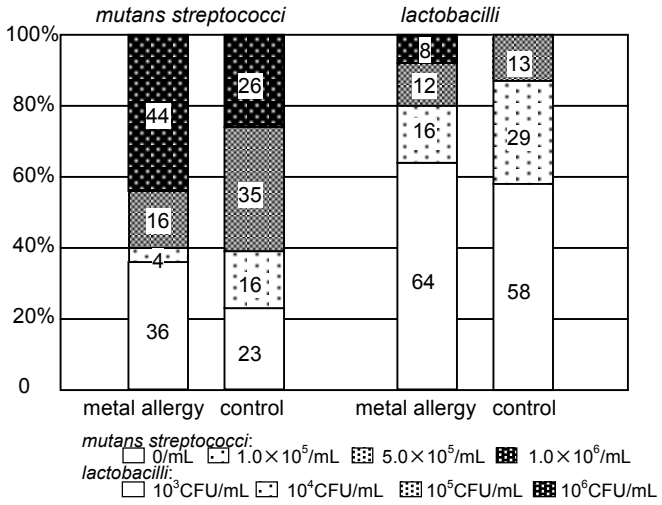

Fig. 2. Counts of *streptococci* and *lactobacilli*.

Concentration of organic acids and carbonic acid in saliva

The mean concentration of organic acids and carbonic acid in the two groups are shown in Table 2. In resting whole saliva, the concentration of pyruvic acid and lactic acid was significantly lower in the metal allergy group than in the control group (p<0.05). In stimulated whole saliva, on the other hand, no significant differences were observed between the two groups (p=0.19-0.85).

Table 2. Concentration of organic acids and carbonic acid in whole saliva in mmol/mL.

	Resting saliva Metal allergy		Resting saliva Control		Stimulated saliva Metal allergy		Stimulated saliva Control	
	Mean	**SD**	**Mean**	**SD**	**Mean**	**SD**	**Mean**	**SD**
Citric acid	3.362	3.362	3.288	1.949	1.696	0.819	1.979	1.639
Pyrubic acid	0.007*	0.016	0.072*	0.092	0.001	0.004	0.007	0.027
Malic acid	0.027	0.046	0.031	0.047	0.024	0.044	0.020	0.041
Succinic acid	0.007	0.024	0.022	0.046	0.049	0.145	0.020	0.034
Lactic acid	0.239**	0.172	0.488**	0.446	0.163	0.168	0.260	0.451
Formic acid	0.299	0.189	0.265	0.199	0.120	0.141	0.127	0.134
Acetic acid	1.426	0.925	1.876	1.195	5.628	15.203	2.084	1.654
Propionic acid	0.120	0.127	0.173	0.245	1.535	5.940	0.266	0.383
Carbonic acid	2.603	9.911	7.908	16.680	34.305	25.707	39.807	36.825

SD=Standard deviation. *, **: Significantly different (p<0.05).

Discussion

With regard to whole saliva, two major differences were noted between the metal allergy group and the control group: 1) the flow rate of resting saliva was lower in the metal allergy group than in the control group; and 2) different concentrations of certain organic acids were found in the resting whole saliva between the two groups.

The flow rate in the two groups, when compared to the figures reported by Ericsson and Hardick, is within normal limits for both resting and stimulated whole saliva.[41,42] The flow rate of resting saliva in the metal allergy group was only 60% that of the rate in the control group. This low flow rate seems to be attributable to the side effects of drugs, since anti-allergic agents, immunosuppressant agents and antihypertensive agents are known to cause dry mouth as a possible side effect.[35,36] However, the comparison of flow rate between participants under medication and those not under medication in the present study revealed no significant difference, indicating that, although drugs may exert an influence on saliva flow rate, this influence was not evident in the present experiment.

With regard to the buffer capacity of saliva and SM and LB counts, no significant difference was observed between the metal allergy group and the control group. The SM count tended to be higher in the metal allergy group, but, with regard to the buffer capacity of saliva and SM and LB counts, the two groups seemed to fulfill all the same conditions.

In the present study, citric, pyruvic, malic, succinic, lactic, formic, acetic, propionic and carbonic acids were found in the resting whole saliva and stimulated whole saliva. The concentrations of acetic, lactic and propionic acids observed in the present study were similar to those reported by Vogel,[43] even though the methods of measurement differed. The concentrations of lactic acid, formic acid, acetic acid and propionic acid observed in the present study are lower than those reported by Linke et al.[29] This disparity may be attributable to the fact that, while Linke et al. used paper point and collected specimens from the molar surface, we collected resting whole saliva including that from the floor of the mouth and obtained stimulated whole saliva by the expectoration method.

Organic acids are thought to be derived from three sources. Food, the first source, contains a wide variety of organic acids.[44,45] The concentration of organic acids in saliva shows a dramatic increase immediately after the ingestion of food, but it has been reported that the concentration gradually decreases and finally returns to the pre-ingestion level after about two hours, leaving behind a certain amount of organic acids in the oral cavity.[25-28] Accordingly, the impact of food can be considered negligible in the present study.[30-32,43]

The second source is organic acids produced by microorganisms in plaque. It has been reported that lactic acid, formic acid and acetic acid, in particular, are produced from piruvic acid derived from the carbohydrate metabolism of microorganisms.[46] Moreover, a great deal of research points to the fact that food ingestion increases the production of organic acids.[30-32,43]

The third source of organic acids is thought to be the ingredients of pure saliva, but uncontaminated saliva must be obtained and analyzed in order to prove this conjecture. We chose whole saliva as the subject of analysis in the present study, because, from the viewpoint of metal allergies, whole saliva contains the corrosive solvents of metal in the oral cavity and remains in contact 24 hours a day with metallic restorations. That is, we chose to analyze whole saliva because the state of whole saliva in the context of daily life correlates most closely with the corrosion of metal ions.

In resting whole saliva, moreover, the concentration of pyruvic acid and lactic acid was lower in the metal allergy group than in the control group. It remains unclear as to whether the concentrations of organic acids observed in the present study are due to morbid characteristics of the participants, differences in the oral hygienic environment, or some other unknown factor. It is also impossible to overrule the influence of drugs on the concentration of organic acids.

A significant difference was observed for resting whole saliva but not for stimulated whole saliva. This is attributed to the different rate of saliva flow from the salivary glands[47,48] and to the differing concentrations of organic acids in parotid saliva, sublingual saliva and submandibular saliva.

The following can also be speculated about the carbonic acids observed in the present study. The carbon dioxide dissolved in saliva when specimens come in contact with air is thought to influence the concentration of carbonic acids.[49-51] A method has been proposed to prevent contact with the atmosphere by collecting specimens under paraffin,[50] but we did not employ this method because it would have impeded our analysis. In a pilot study, moreover, we noted that there was very little difference in the concentration of carbonic acid in saliva immediately after collection and that after the thawing of frozen specimens: the concentration in resting saliva was 9.05 μmol/mL and 8.57 μmol/mL immediately after collection and after thawing, respectively ($p=0.16$, $n=5$; unpublished data). We therefore adopted the face concentration of carbonic acid as the statistical value in the present study. The higher concentration of carbonic acid in stimulated whole saliva as compared to resting whole saliva may be due in part to the longer exposure to air resulting from the chewing of the paraffin pellet. In view of this fact, the authors reserve judgment on the concentration of carbonic acid in the present study.

A considerable amount of research has been conducted on the influence of metal corrosion on lactic acid solution and other individual acids,[21-23] but, to the best of our knowledge, there is no report on metal corrosion in the presence, simultaneously, of the nine acids reviewed in the present study. Our results will facilitate the investigation regarding the safety and bio-compatibility of dental materials under conditions similar to the natural state.

The impact of drugs on the concentration of organic acids in saliva, as well as organic acids not evaluated in the present study, are topics for future research.

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