In this study, patients with heart diseases were classified into 2 groups: Warfarin user and Warfarin non-user, and six salivary components were determined to assess intraoral pathologic conditions. Groups of healthy subjects and patients with periodontal disease without receiving any medication were set as control groups, and they were compared with those of the 2 groups with heart diseases. In patients with heart diseases in both the groups, albumin (ALB) level was found to be significantly higher compared to that in the control groups, and it was significantly higher in the patient group receiving Warfarin user and Warfarin non-user compared to that in the patient group with periodontal disease. C-reactive protein (CRP) levels were found to be higher in both the groups with heart diseases than those in the healthy group. Correlations between various salivary components and the clinical parameters were examined, showing significant correlations between ALB and gingival index (GI) and clinical attachment level (CAL), and between alanine aminotransferase (ALT) and GI, probing depth (PDL), bleeding on probing (BOP) and CAL.

Significant correlations were also found between creatine kinase (CK) and PDL, GI and BOP. Thus, it was suggested that ALB and CRP might serve as the markers of intraoral pathologic conditions, and CK and ALT might serve as those alternative to GI.

Key words: saliva, albumin, C-reactive protein, alanine aminotransferase, creatine kinase

Introduction

Along with the recent diversification of medical care, the number of Warfarin users visiting the dentist is increasing. Warfarin, which is a prophylactic and therapeutic drug for thrombosis and embolism, is indicated to the treatment of postoperative cases of prosthetic valve surgery, and case of myocardial infarction, atrial fibrillation and pulmonary embolism. The event number of the representative treatment, prosthetic valve surgery, reached nearly 7,000/year in 1998 in Japan. Moreover, nearly 100% of these postoperative patients received Warfarin anticoagulation drug therapy, and this therapy is being continued semipermanently.1

One of the most frequently observed adverse reactions of Warfarin is bleeding2,3. Concerning the oral cavity, Warfarin users have gingival bleeding on teeth brushing4 to make them hesitate to brush their teeth. As a consequence, periodontal disease worsens in many

Original Article

Changes in Salivary Components by Drug Administration in Patients with Heart Diseases

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Warfarin users. Although gingival bleeding is relatively mild bleeding, its incidence is high. Thus, it is conceivable that gingival bleeding due to the use of Warfarin makes periodontal disease severer.

On the above background, patients with heart diseases, who were concurrently affected by periodontal disease, were grouped into two categories; one receiving Warfarin and the other receiving drugs other than Warfarin, and several salivary components were analyzed to compare the results with those obtained by the conventional intraoral evaluation methods.

Materials and Methods

1. Subjects
Except for 10 healthy subjects, periodontal disease patients with periodontal pockets deeper than 4 mm were used exclusively as described below.

The subjects groups studied consisted of the following: Group O: 10 healthy subjects without periodontal disease (mean age: 28.2±5.4 years), Group P: 18 patients with periodontal disease (30.1±9.9 years), Group HP: 20 Warfarin non-user patients with heart diseases (65.2±5.9 years), Group WHP: 14 Warfarin users (60.3±12.7 years). Prior to the registration of these subjects, written informed consent to participate in the present investigation was obtained from individual subjects.

2. Collection and pretreatment of whole saliva
For collection of whole saliva, each subject rinsed his oral cavity with water, then chewed PARAFFIN WAX (ORION, Finland) for several minutes and spat it out into a 50 ml CORNING tube (CORNING, USA). In this way, 10 ml of a whole saliva sample was collected from every subject. Immediately after collection, saliva samples were kept in a refrigerator, and then centrifuged at 3,000 rpm for 15 min (Swing-type Centrifuge No. GS-6KR, BECKMAN, USA), and the supernatant was delivered into tubes (1 ml/tube). The tubes were stored frozen at -80°C, thawed on measurement, and analyzed.

3. Biochemical assay methods
Salivary total protein (TP) was determined using HITACHI U-1000 (HITACHI, Japan), using Micro TP-test WAKO (Wako Pure Chemical Industries, Japan) for TP is based on the pirogallol red molibudate method, N-assay TIA Micro Alb (Nittobo Medical, Japan) for ALB is based on immuno turbidity method, ELPiase CRP-H (DIA-IATRON, Japan) for CRP is based on immuno turbidity method, Detaminer AST (KYOWA MEDEX, Japan) for AST, Detaminer ALT for ALT are based on the Karmen method, and Detaminer L CPK (KYOWA MEDEX, Japan) for CK is based enzymatic method. These reagents were used for individual components in serum.

4. Oral cavity evaluation
As parameters for oral cavity evaluation, plaque index (PI) (Silness & Löe), gingival index (GI) (Löe & Silness), probing depth (PD), bleeding on probing (BOP), and clinical attachment level (CAL) were used.

For evaluation of clinical symptoms based on clinical parameters, the above parameters were used or measured in the following manner: 1) PI was used for evaluating the state of dental plaque adhesion. 2) GI was used for evaluating the spreading and severity of gingival margin inflammation. 3) PD was measured by inserting a CPUNC15 probe (HU-FRIEDY, USA) at a pressure of about 25 g to measure the distance from the periodontal pocket bottom to the gingival margin at a unit of 1 mm. 4) CAL was measured as the distance from the periodontal pocket bottom to the cement-enamel junction. 5) BOP was evaluated based on the presence or absence of gingival bleeding on probing.

BOP was scored as 1 when bleeding was observed within 10 to 30 sec after inserting a probe in parallel to the tooth axis into the gingival crevice (periodontal pocket bottom) at a force of 25 g, and it was scored as 0 when no such bleeding occurred.

These measurements 1) to 5) were carried out for residual teeth using the 6 points method, and the mean values from individual subjects were investigated by disease.

5. Statistical analysis
KyPlot for Windows (Kyens Lab. Japan) was used for the statistical analysis. In addition, Kruskal-Wallis test and Steel-Dwass test were used for correlation with the individual salivary components and clinical parameters. All tests were two-tailed and differences were statistically significant at p<0.05 for the null hypothesis.
**Results**

1. Comparison of the mean values of six salivary components in each group (Table 1)

TP wasn't observed no significant difference among 4 groups. ALB was significantly high in Groups HP and WHP compared with Group O (p < 0.01 and p < 0.05, respectively) or Group P (p < 0.01 and p < 0.05, respectively). CRP was significantly high in Group HP and WHP compared with Group O (p < 0.001 and p < 0.01, respectively).

Of AST, ALT and CK, only ALT showed significant differences between Group P and Group WHP (p < 0.05).

2. Comparison of the mean values of clinical parameters in each group (Table 2)

All of PlI, GI, PD, BOP and CAL were significantly high in Group HP and Group WHP compared with Group P. However a significant difference wasn't observed for all clinical parameters between Group HP and Group WHP.

3. Correlation among six saliva components and clinical parameters (Table 3)

Correlations between various salivary components and the clinical parameters were examined, showing significant correlations between ALB and GI and CAL, and between ALT and GI, PD, BOP and CAL. Significant correlations were also found between CK and PlI, GI and BOP.

**Discussion**

Since the platelet function and blood coagulation ability are known to elevate at an early stage after prosthetic valve surgery, it is suggested that not only anti-coagulation therapy but also the concomitant use of anti-platelet drugs are needed for such postoperative patients\textsuperscript{10,11}. In the study of 203 myocardial infarction patients treated with thrombolytic therapy, Warfarin is reported to be superior to the long-term administration of Aspirin for the prevention of recurrent cardiac complications\textsuperscript{12}.

TP and ALB are derived from transudation of serum components into the oral cavity. Although some papers reporting on the salivary levels of TP and ALB are available, the values are variable by the papers\textsuperscript{13,14,15}. Meurman et al. examined in a total of 252 cases of ambulant patients over 70 years in age, and they reported that TP and ALB levels were 1.6±0.8 mg/ml (1,600±800 mg/l) and 204.6±179.5mg/ml (204.6±179.5 mg/l), respectively. On the other hand,
Mellanen et al. reported that TP and ALB levels were $0.7 \pm 0.51$ mg/ml ($700 \pm 510$ mg/l) and $126.4 \pm 54$ mg/l, respectively. Ruhl et al. reported that ALB level was $38.5 \mu$g/ml. Our data of TP and ALB obtained in healthy subjects were $622.6 \pm 223.2$ mg/l and $118.0 \pm 91.1$ mg/l, respectively, being almost consistent with those reported by Mellanen et al. As to TP level, no significant differences were found among all the groups. Whereas, although no significant differences in ALB level were found between Group WHP and Group HP, both the groups with heart diseases showed a significantly higher ALB level compared to that in Group O and Group P. A possibility was suggested that the difference was due to the age difference, because the ages of the subjects in both Group O and Group P were lower compared to those in the heart disease groups. However, if the difference could be attributed solely to the age difference, TP levels must also be different. In addition, salivary flow might be a critical issue, but we consider that it can be neglected in our study because the saliva was obtained by stimulation with paraffin, not by natural secretion. Ferreiro et al. measured the stimulated salivary flow of the whole saliva in patients with chronic hepatitis C, showing that it was independent of age, gender, as well as AST and ALT levels. In the present study, samples of saliva could not be obtained from elderly subjects corresponding to patients with heart diseases without being affected by periodontal disease; therefore we had no choice but to compare the data for different age groups. In the present study, we focused only on patients with heart diseases, but it was considered necessary to compare the data obtained in patients with periodontal disease complicated by other diseases. Cinquins et al. measured the salivary levels of AST, ALT and LD in type I diabetic children, and they reported that a significantly high level of any of these enzymes was found only in children with a morbid period shorter than 4 years. They attributed the cause of this finding to transudation of these enzymes from the salivary gland cells. Cesco et al. found that a patient group with a high severity class of C4 according to the classification by the CPITN (Community Periodontal Index of Treatment Needs) showed a significantly high AST level. They used the Reflotron$^\text{TM}$ system for determining serum AST levels, and reported

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* p < 0.05  ** p < 0.01  *** p < 0.001
that a patient group of CO (healthy subjects group) showed a mean value of 30.9 U/ml with an inter quartile range of 14.7 to 41.7 U/ml. In addition, Hisano A. et al. also reported a value of 37.7 U/l in a healthy subject group. Our data were found to be lower compared to these values, being about 50% of the reported data on average. On enzyme assay, enzyme activities are variable depending on the measurement temperature, substrate concentration, the presence or absence of coenzymes, etc; therefore it may be difficult to compare the data with those reported in other papers. In our present study, although there was no significant difference in AST, a trend of high level was found, which was consistent with the reported results. As to ALT, there was a significant difference between Group WHP and Group P. It was notable that a different pattern was only found for ALT between Group WHP and Group HP.

As to CK, Petrovich et al. reported that it increased in patients with periodontal disease. CK was showed high in the mean values of Group HP and Group WHP compared with Group O and Group P, but no significant difference among 4 groups in our data. Christodoulides et al. measured salivary CRP levels. They newly constructed a high-sensitivity microchip assay system for determination of salivary CRP, with which the analysis can be carried out by diluting the saliva up to 100-fold. They found that blood CRP level increased in patients with cardiovascular disease (CVD), and that CVD was accompanied by oral infection such as periodontal disease. From these findings, they concluded that CRP was the best marker. We determined CRP using a high-sensitivity CRP reagent for serum, and found that both the patient groups with heart disease showed a high CRP level compared to that in Group O. This was considered to be the characteristics of heart disease group, because no significant differences were found between Group P and Group O.

We examined the correlation between salivary components and the intraoral evaluation parameters. When it was examined for all the patient groups, ALB, ALT and CK showed a significant correlation with GI. GI is considered to be a parameter reflecting the severity and extension of inflammation to the gingival periphery, which indicates intraoral inflammation as suggested by Cinquini et al., who reported that AST, ALT and LD were transuded from the damaged cells of the salivary grand. In particular, it was notable that the data obtained in the present study showed a significant correlation in CK among the all patient group, Group P, Group HP and Group WHP. BOP showed a significant correlation with ALT and CK. BOP is a parameter of gingival bleeding, therefore the correlation is considered to be the same as that of GI with ALT and CK. However, no significant differences were found for ALB, but it showed a significant correlation with CAL, a parameter reflecting bone absorption. Although Hisano et al. reported that a significant correlation was found between BOP and AST (p<0.05), our data showed a significant correlation between BOP and AST only in Group HP.

## Conclusions

Salivary TP, ALB, CRP, AST, ALT and CK were determined in patients with heart diseases classifying them into Warfarin user and Warfarin non-user group. As a result, there were no significant differences in these laboratory data between the two groups. However, there were many cases showing a high ALT level in Warfarin user group. Patients with heart diseases showed high salivary levels of ALB and CRP. In the correlation between salivary components and the intraoral evaluation parameters, CK and ALT showed a good correlation with GI.

## Acknowledgements

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## References