Prevalence of blood contamination in adult saliva and quantitative measurement of salivary hemoglobin level by using an anti-human hemoglobin monoclonal antibody

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Purpose: Blood contamination in saliva is more common in individuals with periodontal destruction. This study aimed to determine the prevalence of salivary hemoglobin and periodontal pathogens in Japanese adults.
Materials and Methods: The study participants were 249 subjects older than 20 years who participated in an oral health promotion festival organized by the Tokyo Minato-ku Shiba Dental Association. We collected 249 saliva specimens from the 249 subjects in the middle of Tokyo, Japan. For bacterial detection, 24 specimens were selected. *Porphyromonas gingivalis* and *Aggregatibacter actinomycetemcomitans* were detected in 24 specimens by using polymerase chain reaction.
Results: The salivary human hemoglobin (hHb) levels in 94 subjects (37.8%) were lower than the detectable level (<1 μg/mL). The remaining 155 subjects (62.2%) were suspected of having periodontal disease. If we adopt a cutoff hHb level of 2.0 μg/mL, 101 subjects (40.6%) would be positive for periodontal disease. Of the 24 saliva specimens, 21 (87.5%) and 3 (12.5%) tested positive for *P. gingivalis* and *A. actinomycetemcomitans*, respectively.
Conclusion: Our data indicate that screening tests for blood contamination in adult saliva were useful for oral and general health.

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Key Words: anti-human hemoglobin monoclonal antibody, hemoglobin, periodontal disease, saliva

Introduction

Martí et al. [1] studied a population of 184 adult patients composed of 101 totally edentulous patients with no oral mucosa lesions and 83 dentulous patients with clinically healthy gingival tissues. Of the dentulous patients, 67% tested positive for hemoglobin contamination in saliva. On the other hand, none of the patients in the totally edentulous group exhibited hemoglobin in saliva. These data suggest that periodontal conditions are an essential factor for the presence of hemoglobin contamination in saliva. In healthy humans, the epithelial barrier provides the first line of defense against the invasion of pathogenic and commensal bacteria. Periodontal pathogens, smoking, and biting force induce inflammatory responses that lead to the destruction of the oral epithelial barrier. As a result of the inflammatory responses, saliva can often be contaminated with hemoglobin. Hemoglobin contamination in saliva is more common in individuals with periodontal destruction. Hemoglobin is a protein composed of four protein chains containing an iron atom. The iron atom is specifically removed from the hemoglobin molecule and used by the periodontal pathogens for their growth.

A new method of immunological detection of hemoglobin was reported in 1991 [2]. The detectable hemoglobin concentration with this method was 0.5 μg/mL. Nagata and Tanaka [3] produced monoclonal antibodies against human hemoglobin (hHb). They analyzed 785 fecal specimens by using colloidal gold agglutination and compared the results with those obtained using latex agglutination. Hemoglobin was detected

in 75 specimens by using colloidal gold agglutination, in 76 specimens by using latex agglutination, and in 70 specimens by using both methods. The overall agreement between the two methods was 98% [3].

In this study, we applied the method for immunological detection of hemoglobin in fecal specimens to saliva specimens, and report the prevalence of salivary hemoglobin and periodontal pathogens in Japanese adults.

Materials and Methods

Participant recruitment

The Tokyo Minato-ku Shiba Dental Association holds annual festivals as oral health promotion efforts to improve oral health through saliva screening. The participants of this study were 249 subjects older than 20 years who participated in an oral health promotion festival in response to recruitment materials (public bulletins, posters, flags, and solicitations).

Saliva specimens

We collected 249 saliva specimens from subjects of various ages, both sexes, and occupation in the middle of Tokyo, Japan, in May 2013. A standardized bolus of a gum base containing neither fragrant nor taste ingredients was given to the subjects. Stimulated whole saliva was collected by chewing on the gum base for 5 minutes.

Measuring salivary hemoglobin

For the examination of salivary hemoglobin levels, the collected saliva specimens were maintained at 4℃. These specimens were immediately transported to the examination laboratory. Salivary hHb levels were measured by using the NS-Plus automated analyzer and saliva immunochemical testing system (Ness Coat Saliva Hemo Plus, Alfresa Pharma Co., Osaka, Japan). Ness Coat Saliva Hemo Plus was used as a diagnostic device for quantitative detection of the hHb in human saliva by using the immunochemical method. Colloidal gold labeled with monoclonal antibody against hHb was agglutinated with hemoglobin and changed its color from red to gray. The color that diminished with the presence of an antigen was unchangeable from 30 min to 4 h. The detectable hemoglobin concentration with this method was 1.0 μg/mL.

Polymerase chain reaction

The polymerase chain reaction kits for detection of periodontal pathogens in saliva were purchased from a medical examination company (Genesis Healthcare, Co., Tokyo, Japan). *Porphyromonas gingivalis* and *Aggregatibacter actinomycetemcomitans* were detected by using PCR.

Ethical considerations

The study was approved by the ethics committee of Tsurumi University School of Dental Medicine (approval No. 430) and conducted in accordance with the Declaration of Helsinki.

Results

Finally, 249 saliva specimens were examined. The distribution of the salivary hHb levels is shown in Fig. 1. One specimen with outlier values that remarkably differed from other data had an hHb level of 876.0 μg/mL. This sample was excluded for the following analysis.

The salivary hHb levels in 94 subjects (37.8%) were <1.0 μg/mL. If we adopt a cutoff hHb level of 1.0 μg/mL, 155 subjects (62.2%) would be suspected as having periodontal disease. Meanwhile, if we adopt a cutoff hHb level of 2.0 μg/mL, 101 subjects (40.6%) would test positive. Bacterial pathogens are important targets for detection and identification in medicine. Saliva specimens that tested positive for *P. gingivalis* and *A.*

actinomycetemcomitans were assessed qualitatively for comparison with different salivary hHb levels.
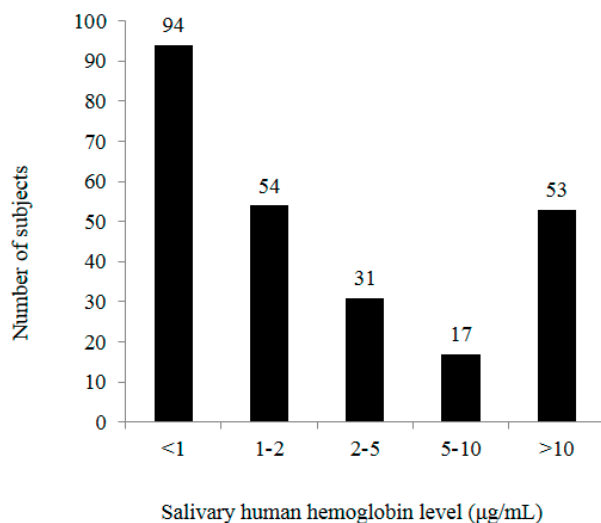

Fig. 1
Distribution of the salivary human
hemoglobin levels

Table 1   Cross tabulation of the presence of periodontal bacteria according to salivary human hemoglobin level

Salivary human hemoglobin level	*Porphyromonas gingivalis*		*Aggregatibacter actinomycetemcomitans*	
(μg/mL)	+	−	+	−
0.1-0.2 μg/mL	11	1	1	11
>80 μg/mL	10	2	2	1

Of the 24 specimens, 21 (87.5%) and 3 (12.5%) tested positive for *P. gingivalis* and *A. actinomycetemcomitans*, respectively (Table 1). The overall detection rate of *P. gingivalis* was high (21/24 specimens), whereas that of *A. actinomycetemcomitans* was low (3/24 specimens). Owing to the extremely wide distribution of the values, we could not find any significant difference between the two groups of salivary hemoglobin levels in terms of the prevalence of periodontal bacteria.

Discussion

Traditional screening methods for periodontal diseases that involve the use of periodontal probing may be uncomfortable or even painful for subjects. In this study, a new method for immunological detection of occult blood in saliva was introduced that used colloidal gold labeled with anti-hHb monoclonal antibody.

If the barrier between the bloodstream and oral mucosa is compromised by chronic inflammation such that blood or plasma leaks into the saliva, then higher levels of biomarkers in the bloodstream could contaminate saliva specimens. Among the blood or plasma biomarkers we tested, salivary hemoglobin and lactate dehydrogenase levels correlated with periodontal destruction conditions [4,5]. These biomarkers also showed an association when the community periodontal index (CPI) method, which involves the use of periodontal probing, was used [4].

In this study, the salivary hHb levels in 94 subjects (37.8%) were lower than the detectable level (<1 μg/mL), which indicated that 37.8% of the subjects had no oral inflammation. This means that 62.2% of the subjects had some inflammation that led to periodontal disease. If we adopt a cutoff hHb level of 2.0 μg/mL, 101 subjects

(40.6%) would test be suspected of having periodontal disease.

By contrast, salivary bacterial measurement was difficult to use for the periodontal disease screening test. The detection rate of *P. gingivalis* was 87.5% in total. In addition, the detection rate of *P. gingivalis* in the subjects with salivary hHb levels within 0.1-0.2 μg/mL was 91.6%. These results indicate that most of the adults had *P. gingivalis* infection. *P. gingivalis* is a well-known bacterium that causes periodontal diseases. To promote oral health and periodontal disease prevention, subjects should undergo eradication therapy for oral cavity *P. gingivalis* infections. Salivary hHb levels of >1 μg/mL should be reduced to below detectable levels (<1 μg/mL) by tooth cleaning for prevention and interrupting the progression of periodontal disease.

A new therapy for dental biofilm management was established by our research group [6-10]. This therapy was named 3DS (Dental Drug Delivery System) and validated by in vivo studies and clinical evaluations. In the 3DS method, antibacterial agents are applied by using individual trays at chair-side and as home-care program. By using individual trays, the dental biofilm that leads to gingivitis is controlled without changing the core composition of the oral microflora. We recommend applying this method in dental clinics worldwide.

Salivary screening has many advantages over other methods. However, mass screening is time-consuming and entails high costs in terms of employment of dentists. Furthermore, before beginning the screening for gingivitis and/or periodontitis, evaluation by the examiner is required. Mass screening using a questionnaire method is simple and inexpensive but not sensitive for periodontal diseases. The progress of periodontal diseases is difficult to determine because they are typically clinically silent. Screening tests for fecal occult blood is popular worldwide. Similarly, screening tests for blood contamination in adult saliva should become popular in the future.

Piazza et al. determined blood contamination in the saliva of patients with acquired immunodeficiency syndrome (AIDS)-related complex/AIDS [11]. Patients with AIDS-related complex/AIDS have a mean salivary hHb level of 190 μg/mL, which corresponds to 1.3 μL of blood per milliliter of saliva. If 10 mL of saliva is exchanged between partners during kissing, an average of 13 μL of blood is transferred [11]. Piazza et al. also detected blood contamination in the saliva of patients with human immunodeficiency virus (HIV) infection [12]. The data show that the anti-HIV-positive group had higher hHb concentrations than the control group. These data indicate that screening tests for blood contamination in adult saliva are necessary for prevention not only of periodontal diseases but also of HIV/AIDS.

The reagent kits for measuring salivary hHb levels (provided by Alfresa Pharma Co.) that were used in this study were approved according to the Pharmaceutical Affairs Law of Japan as official in vitro diagnostic reagent for the evaluation of the periodontal conditions. This approval is a great advantage of salivary hHb screening over other biomarker tests for periodontal diseases, which have not been approved according to the law.

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