



OPEN

Development of a novel method to evaluate gingivitis in children by combining rapid saliva test systems

Yuko Iwamoto^{1,3}, Chieko Mitsuhasha^{1,3}, Shunya Ikeda¹, Yuya Ito¹, Shuma Hamaguchi¹, Eimi Tabata¹, Yasuko Tsuge¹, Ami Kaneki¹, Masashi Ogawa¹, Taku Nishimura¹, Mariko Kametani¹, Satoru Kusaka², Yuria Asao¹, Tatsuya Akitomo¹, Katsuyuki Koza¹ & Ryota Nomura¹✉

Few tools can objectively assess the risk of gingivitis in children. We developed a novel method to evaluate gingivitis by combining rapid saliva test systems. Seventy-six subjects (aged 3–19 years) were included in the study. Gingival condition was evaluated using the papillary marginal attached (PMA) index. Liquid gargled by the subjects was examined using Perioscreen to measure the amount of hemoglobin, and the Salivary Multi Test (SMT) to quantify six factors related to oral health. PMA values were significantly higher in subjects with positive hemoglobin than in those with negative hemoglobin ($P < 0.001$). Of the six SMT items, buffering capacity and protein showed correlations with PMA value ($R^2 > 0.25$). Next, a multivariate receiver operating characteristic (ROC) analysis for the diagnosis of gingivitis was performed using PMA value as the outcome and hemoglobin, buffering capacity, and protein as variables. The results showed that the area under the curve (AUC) for the combination of the three variables, including hemoglobin, buffering capacity, and protein, was significantly higher than when any single variable was used ($P < 0.05$). Our findings suggest that this new method could identify children at risk of gingivitis by complementing hemoglobin detection with protein and buffering capacity detection.

The prevalence of gingivitis in children is high, affecting half of the population by age 4–5 years, and some reports suggest that the prevalence reaches nearly 100% by adolescence¹. However, periodontitis in children may be associated with systemic or immune disorders, or may result in aggressive periodontitis of unknown etiology, but these are quite rare^{1,2}. According to many periodontists, bleeding on probing is the most reliable method for assessing a patient's gingival status in clinical practice³. Once children can cooperate with bleeding on probing, it is recommended that they begin regular probing to detect early signs of periodontal disease and prevent its progression.

Gingivitis in children is frequently observed, mainly due to poor oral hygiene. Saliva is an effective tool for objectively evaluating biological data related to health, including information about the oral cavity, which can also be used to evaluate gingivitis^{4,5}. Liquid after gargling can be collected more easily than saliva, making it particularly useful when testing children. However, few studies have attempted to evaluate the state of gingivitis in children using liquid after gargling.

Testing methods for periodontal disease include kits that can detect blood-derived hemoglobin from inflamed gingiva in saliva or liquid after gargling^{6,7}. Hemoglobin can hold oxygen in an aerobic environment (oxy form), while hemoglobin without oxygen in an anaerobic environment (deoxy form) has a high buffering capacity⁸. Current hemoglobin detection kits are accurate and highly sensitive, but they only allow quantitative assessment of hemoglobin.

The recently developed Salivary Multi Test (SMT; Lion Dental Products Co. Ltd, Tokyo, Japan) uses saliva or liquid after gargling to obtain multiple items of information about oral health in a short time⁹. The SMT can measure six items: cariogenic bacteria, acidity, buffering capacity, white blood cells, protein, and ammonia. Cariogenic bacteria, acidity, and buffering capacity are used as evaluation items for dental caries risk, white

¹Department of Pediatric Dentistry, Graduate School of Biomedical and Health Sciences, Hiroshima University, -2-3 Kasumi, Minami-Ku, Hiroshima 734-8553, Japan. ²Department of Pediatric Dentistry, Hiroshima University Hospital, Hiroshima, Japan. ³Yuko Iwamoto and Chieko Mitsuhasha have equally contributed to this work. ✉email: rnomura@hiroshima-u.ac.jp

blood cells and protein are used as evaluation items for periodontal condition, and ammonia is used as an evaluation item for oral hygiene⁹. The SMT is easy to apply to young children who are able to gargle¹⁰.

In the present study, we developed a novel method for specifically and simply assessing the risk of gingival inflammation in children by combining a hemoglobin detection kit and six items from the SMT, using liquid after gargling.

Results

Subjects

Subjects were 76 children (50 boys and 26 girls) who visited the Pediatric Dentistry Department of Hiroshima University Hospital. The subjects ranged in age from 3 to 19 years, with a mean age of 8.5 ± 3.9 years (mean age \pm standard deviation) and a median age of 7 years.

Relationship between hemoglobin concentration and PMA index

Gingival inflammation of the maxillary anterior teeth was evaluated using PMA index. Each region was assigned a score of 1 for the presence of inflammation and 0 for the absence of inflammation, with the sum of these scores calculated as the maximum of 34. Subjects' scores ranged from 0 to 24 points, with the distributions being 0 (21.1%), 1–5 (28.9%), and 6–10 (30.3%), with less than 10% of subjects scoring 11 or more points (Fig. 1A).

Hemoglobin levels were measured in the liquid after gargling using Perioscreen, and the percentages of subjects with hemoglobin concentrations of 0 $\mu\text{g}/\text{mL}$, 2 $\mu\text{g}/\text{mL}$, and 5 $\mu\text{g}/\text{mL}$ were distributed between 30 and 40% (Fig. 1B). The PMA values were significantly higher in subjects with hemoglobin concentrations of 2 $\mu\text{g}/\text{mL}$ ($P=0.0010$) and 5 $\mu\text{g}/\text{mL}$ ($P<0.0001$) compared to subjects with a hemoglobin concentration of 0 $\mu\text{g}/\text{mL}$ (Fig. 1C).

Relationship between SMT items and PMA index

The liquid collected after gargling was analyzed using an SMT device to quantify six factors (cariogenic bacteria, acidity, buffering capacity, white blood cells, protein, and ammonia), each with a minimum score of 0 and a maximum score of 100. The score ranges for each parameter were as follows (minimum score—maximum score, mean \pm standard error): cariogenic bacteria 0–60, 20.0 ± 2.0 ; acidity 0–100, 55.6 ± 2.6 ; buffering capacity 0–70, 20.9 ± 1.7 ; white blood cells 0–79, 33.9 ± 2.6 ; protein 0–88, 38.7 ± 2.0 ; and ammonia 11–100, 45.0 ± 2.2 . The data from the regression analysis between the PMA index and SMT items are shown in Table 1. The slope of the correlations between the PMA index and SMT items showed significant differences for four of the six SMT items (acidity, buffering capacity, protein, and ammonia) ($P<0.05$) (Figs. 2A and 3F). Of these, buffering capacity and protein had a coefficient of determination of 0.25 or more.

Relationship between hemoglobin concentration and SMT items

Next, we analyzed the correlation between the hemoglobin concentration obtained from the Perioscreen and the six SMT items (Figs. 3A–F). The item of protein increased depending on the hemoglobin concentration and there were significant differences between each group (0 $\mu\text{g}/\text{mL}$ vs 2 $\mu\text{g}/\text{mL}$ $P=0.0347$; 0 $\mu\text{g}/\text{mL}$ vs 5 $\mu\text{g}/\text{mL}$ $P<0.0001$; 2 $\mu\text{g}/\text{mL}$ vs 5 $\mu\text{g}/\text{mL}$ $P=0.0151$). However, the other five items did not differ significantly between each hemoglobin concentration. There was a significant difference between buffering capacity and protein in the linear trend test (buffering capacity $P=0.0146$; protein $P<0.0001$).

Comparison of PMA index, hemoglobin concentration and SMT items between each age group

The subjects were divided into the following groups based on age (age range, mean age \pm standard deviation); preschoolers (3–5 years, 4.4 ± 0.7 years) ($n=18$), middle childhood (6–12 years, 8.1 ± 2.0 years) ($n=44$), and adolescents (13–19 years, 15.0 ± 1.8 years) ($n=14$). Data for each age group regarding evaluation items related to oral health are shown in Table 2. Gingival inflammation measured using the papillary marginal attached (PMA) index was significantly higher in the middle childhood ($P=0.0010$) and adolescent groups than in the preschoolers ($P=0.0001$). In addition, the white blood cells and protein levels in the liquid after gargling with Perioscreen (Sunstar Inc., Osaka, Japan) or SMT were significantly higher in the middle childhood group than in the preschoolers (white blood cells $P=0.0498$; protein $P=0.0451$). Furthermore, the hemoglobin, buffering capacity, and protein levels in the liquid after gargling with Perioscreen or SMT were significantly higher in the adolescent group than in the preschoolers (hemoglobin $P=0.0104$; buffering capacity $P=0.0063$; protein, $P=0.0007$).

Receiver operating characteristic (ROC) analysis for the diagnosis of gingival inflammation

The hemoglobin concentration as measured by the Perioscreen and the two SMT items of buffering capacity and protein showed correlations with the PMA index; we decided to focus on these for further analysis. We performed receiver operating characteristic (ROC) analysis using hemoglobin, buffering capacity, and protein as explanatory variables, with PMA values as the outcome (low PMA score group (0–5, $n=38$) and high PMA score group (6–24, $n=38$)). For hemoglobin, a cutoff value of ≥ 1 obtained using the Youden index yielded a sensitivity of 0.947, specificity of 0.553, and area under the curve (AUC) of 0.787 (Fig. 4A). For buffering capacity, a cutoff value of ≥ 21 yielded a sensitivity of 0.658, specificity of 0.789, and AUC of 0.744 (Fig. 4B). For protein, a cutoff value of ≥ 36 yielded a sensitivity of 0.763, specificity of 0.737, and AUC of 0.780 (Fig. 4C).

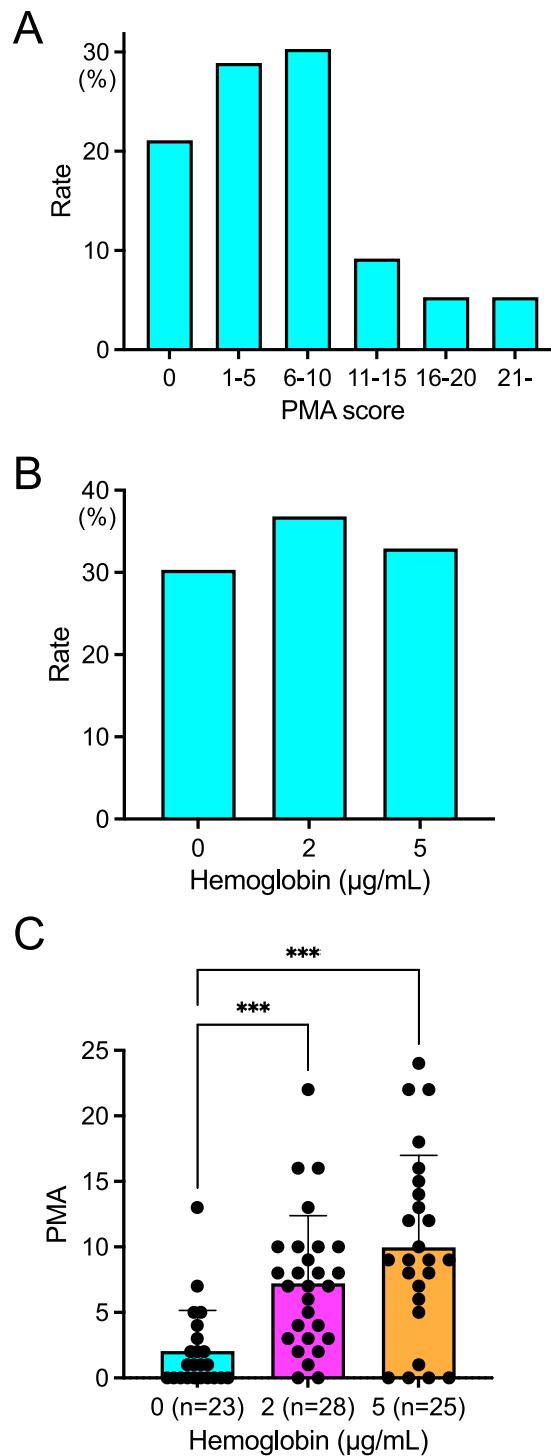


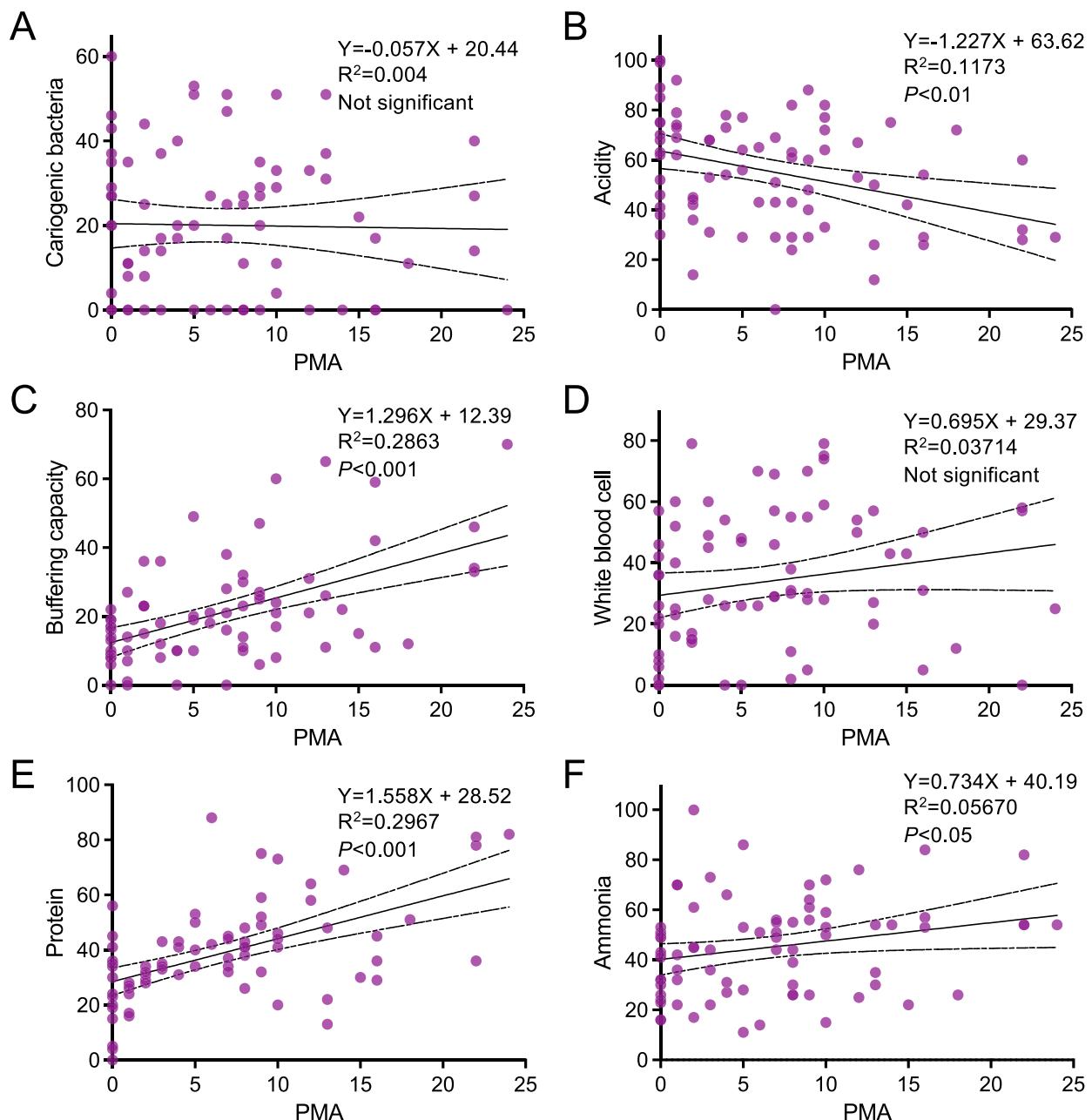
Fig. 1. Distribution of papillary marginal attached (PMA) values and hemoglobin concentrations by Perioscreen and their relationships. (A) Distribution of PMA values. (B) Distribution of hemoglobin concentrations. (C) Relationship between PMA value and hemoglobin concentration. Each circle represents the data of a subject. Bars indicate the mean PMA score. Data expressed as the mean \pm standard deviation. *** $P < 0.001$ between the groups.

Relationship between hemoglobin concentration, SMT items, and PMA index

The items of buffering capacity and protein were divided into two groups based on the median value of all subjects (buffering capacity ≤ 17 and ≥ 18 , protein ≤ 35 and ≥ 36). Hemoglobin concentration was divided into two groups, with $0 \mu\text{g/mL}$ as negative and $2 \mu\text{g/mL}$ and $5 \mu\text{g/mL}$ as positive.

In the analysis focusing on hemoglobin and buffering capacity, the hemoglobin-positive / high buffering capacity group (≥ 18) had significantly higher PMA values than both of the hemoglobin-negative groups

SMT item	r value	Regression coefficient	Standard error	95% confidence interval	P value
Cariogenic bacteria	-0.063	-0.057	0.322	-0.699, 0.585	0.8608
Acidity	-0.343	-1.227	0.391	-2.01, 0.447	0.0025
Buffering capacity	0.535	1.296	0.238	0.822, 1.770	<0.0001
White blood cells	0.193	0.695	0.411	-0.125, 1.510	0.0953
Protein	0.545	1.558	0.279	1.000, 2.110	<0.0001
Ammonia	0.238	0.734	0.348	0.041, 1.427	0.0383

Table 1. Data from the regression analysis between the PMA index and SMT items.**Fig. 2.** Relationship between papillary marginal attached (PMA) value and Salivary Multi Test (SMT) items. SMT items: cariogenic bacteria (A), acidity (B), buffering capacity (C), white blood cells (D), protein (E), and ammonia (F). Each circle represents the data of a subject.

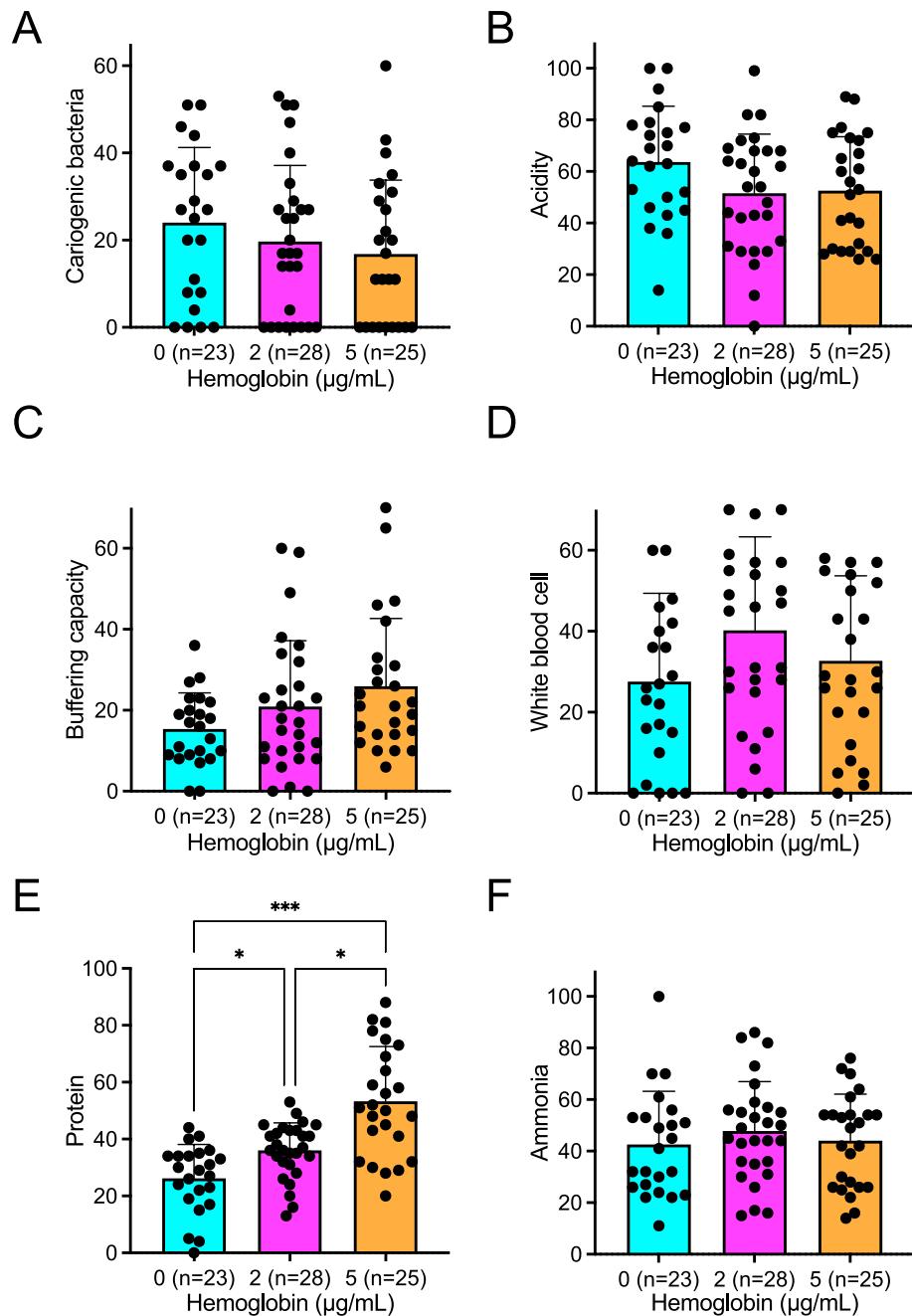


Fig. 3. Relationship between the Perioscreen hemoglobin concentration and Salivary Multi Test (SMT) items. SMT items: cariogenic bacteria (A), acidity (B), buffering capacity (C), white blood cells (D), protein (E), and ammonia (F). Each circle represents the data of a subject. Bars represent the mean SMT value. Data expressed as the mean \pm standard deviation. $^*P < 0.05$ and $^{***}P < 0.001$ between the groups.

(buffering capacity ≤ 17 $P < 0.0001$; buffering capacity ≥ 18 $P = 0.0028$) and the hemoglobin-positive / low buffering capacity group (≤ 17) ($P = 0.0276$) (Fig. 5A). In the analysis focusing on hemoglobin and protein, the hemoglobin-positive / high protein group (≥ 36) had significantly higher PMA values than the hemoglobin-negative / low protein group (≤ 35) ($P < 0.0001$) (Fig. 5B). Regarding the relationship between buffering capacity and protein, the high buffering capacity / high protein group had significantly higher PMA values than the low buffering capacity / low protein group ($P < 0.0001$) and the high buffering capacity / low protein group ($P = 0.0057$) (Fig. 5C).

We then focused on the three items of hemoglobin, buffering capacity, and protein. The group that was hemoglobin-positive and with high values for both buffering capacity and protein had significantly higher PMA values than the groups that were hemoglobin-negative and with low values for protein (buffering capacity ≤ 17 $P < 0.0001$; buffering capacity ≥ 18 $P = 0.0041$), and the group that was hemoglobin-positive and with low values for both buffering capacity and protein ($P = 0.0418$) (Fig. 5D).

Test name	Test item	3–5 (n = 18)	6–12 (n = 44)	13–19 (n = 14)	Total (n = 76)
PMA	Gingival inflammation	1.9 ± 0.7	7.2 ± 0.9**	10.4 ± 1.8***	6.6 ± 0.7
Perioscreen	Hemoglobin	0.7 ± 0.2	1.0 ± 0.1	1.5 ± 0.2*	1.0 ± 0.1
Salivary multi test	Cariogenic bacteria	19.2 ± 3.9	20.2 ± 2.6	20.6 ± 5.1	20.1 ± 2.0
	Acidity	66.2 ± 4.4	51.0 ± 3.3	56.2 ± 6.4	55.6 ± 2.6
	Buffering capacity	14.0 ± 2.6	20.8 ± 2.1	29.9 ± 4.8**	20.9 ± 1.7
	White blood cells	23.6 ± 4.5	38.7 ± 3.2*	32.1 ± 7.0	33.9 ± 2.6
	Protein	26.8 ± 3.7		49.6 ± 4.1***	38.7 ± 2.0
	Ammonia	41.3 ± 3.9	44.8 ± 3.0	50.5 ± 5.3	45.0 ± 2.2

Table 2. Test data for each age group. Data are expressed as mean ± standard error. There were statistically significant differences compared to 3–5-year-old group (*P < 0.05, **P < 0.01, ***P < 0.001).

Multivariate ROC analysis for the diagnosis of gingival inflammation

We performed a multivariate ROC analysis based on a logistic regression model, combining variables including hemoglobin, buffering capacity, and protein, with PMA score as the outcome (Table 3, Supplementary Fig. 1). The AUCs for hemoglobin and buffering capacity, hemoglobin and protein, and buffering capacity and protein were 0.848, 0.834, and 0.819, respectively, and all of which had higher AUCs than models using any single variable (0.744–0.787). Furthermore, the AUC for the combined hemoglobin, buffer capacity, and protein variables was 0.856, which was significantly higher than the AUC for any single variable (vs hemoglobin $P = 0.035$; vs buffering capacity $P = 0.032$; vs protein $P = 0.050$).

Discussion

Kits that can test for blood-derived hemoglobin in gingival crevicular fluid using saliva or liquid after gargling have been used mainly in adults^{6,7}. Recent studies in adults have reported that detection of hemoglobin in gingival crevicular fluid can be a useful marker for detecting subtle damage to periodontal tissues that may be a sign of the onset of periodontal disease, even in cases where bleeding on probing is not a problem¹¹. We aimed to analyze whether hemoglobin in gingival crevicular fluid can be detected in children, who have fewer teeth than adults, shallower gingival sulci, and little severe periodontitis. Our results show that the Perioscreen detected 2 µg/mL or 5 µg/mL of hemoglobin in the liquid after gargling of approximately 70% of children. The PMA values were significantly higher in hemoglobin-positive subjects than in hemoglobin-negative subjects, suggesting that this is a simple and effective method for screening gingival inflammation.

Unexpectedly, the SMT item of buffering capacity, which is a criterion for evaluating dental caries, positively correlated with the PMA value. Hemoglobin has a buffering capacity, and structurally, hemoglobin that does not hold oxygen (deoxy form) has a higher buffering capacity than hemoglobin that holds oxygen (oxy form)⁸. In the present study, correlations were found between hemoglobin and the PMA value, and between buffering capacity and the PMA value. These results suggest that the buffering capacity value of the SMT index can include hemoglobin leaked from periodontal tissues. It is possible that the buffering capacity value increases when hemoglobin increases quantitatively due to periodontal tissue destruction or when hemoglobin qualitatively changes to the deoxy form under anaerobic conditions in the periodontal pocket.

The SMT items of white blood cells and protein were used to evaluate periodontal status⁹. There was no correlation between white blood cells and PMA value. White blood cells, including neutrophils, macrophages, B cells, and substances released from these cells, are activated by infection with highly pathogenic bacteria such as *Porphyromonas gingivalis*, causing inflammation and progression of periodontitis^{12–15}. Unlike adults, children rarely suffer from periodontitis, and highly pathogenic periodontal bacteria are rarely detected^{16–18}. In the present study, none of the subjects had severe periodontitis, and it is possible that there was little induction of white blood cells associated with bacterial infection in the periodontal tissues.

Proteins in the gingival crevicular fluid are biochemical indicators that increase in the early stages of periodontal disease¹⁹. Gingival crevicular fluid contains proteins such as defensins, annexins, transferrin, lactoferrin, and albumin^{20,21}. In the present study, the SMT item of protein correlated with PMA value, and it became clear that protein measurement is effective for evaluating gingivitis in children. Additionally, the Perioscreen hemoglobin value correlated with the SMT item of protein, possibly because hemoglobin is the main iron-containing protein in blood²². SMT protein was detected when no hemoglobin was detected by the Perioscreen probe, suggesting that the SMT detects proteins other than hemoglobin in gingival crevicular fluid.

ROC analysis allows for visual and statistical understanding of diagnostic accuracy and is widely used in the medical field^{23,24}. The results of the ROC analysis suggested that hemoglobin, buffering capacity, and protein may all be valuable biomarkers for identifying gingivitis. Hemoglobin has a high sensitivity (0.947), making it useful for screening purposes; however, its low specificity (0.553) may lead to false positives. Buffering capacity has a relatively high specificity (0.789), potentially contributing to improved positive predictive value, but the somewhat low sensitivity (0.658) means caution is advised when using it alone. Protein exhibited a good balance between sensitivity (0.763) and specificity (0.737), demonstrating its usefulness as a diagnostic indicator for gingivitis.

When the influence of the SMT items of buffering capacity or protein on the PMA index was analyzed in combination with the Perioscreen hemoglobin, the PMA value was significantly higher in the high buffering

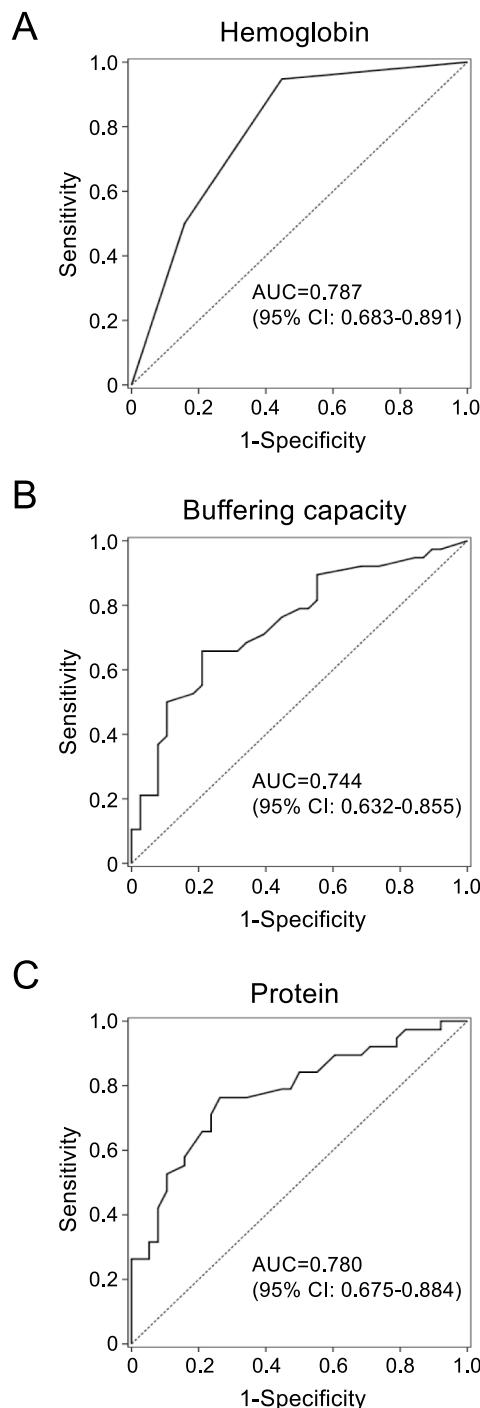


Fig. 4. Receiver operating characteristic (ROC) curves for the identification of gingivitis using each evaluation method. (A) Hemoglobin: area under the curve (AUC) = 0.787, cutoff value ≥ 1 , sensitivity = 0.947, specificity = 0.553. (B) Buffering capacity: AUC = 0.744, cutoff value ≥ 21 , sensitivity = 0.658, specificity = 0.789. (C) Protein: AUC = 0.780, cutoff value ≥ 36 , sensitivity = 0.763, specificity = 0.737.

capacity group than in the low buffering capacity group in the hemoglobin-positive group. Additionally, the PMA value was significantly higher in the high protein group than in the low protein group in the hemoglobin-positive group. These results suggest that by combining the Perioscreen and the SMT, in addition to the existing quantitative evaluation for hemoglobin^{6,7}, it is possible to qualitatively evaluate hemoglobin by focusing on buffering capacity, and to detect a variety of proteins. Furthermore, when subjects were divided into groups based on the three items of hemoglobin, buffering capacity, and protein, the group that was hemoglobin-positive with high buffering capacity and high protein had the highest PMA values. These results indicate that determining

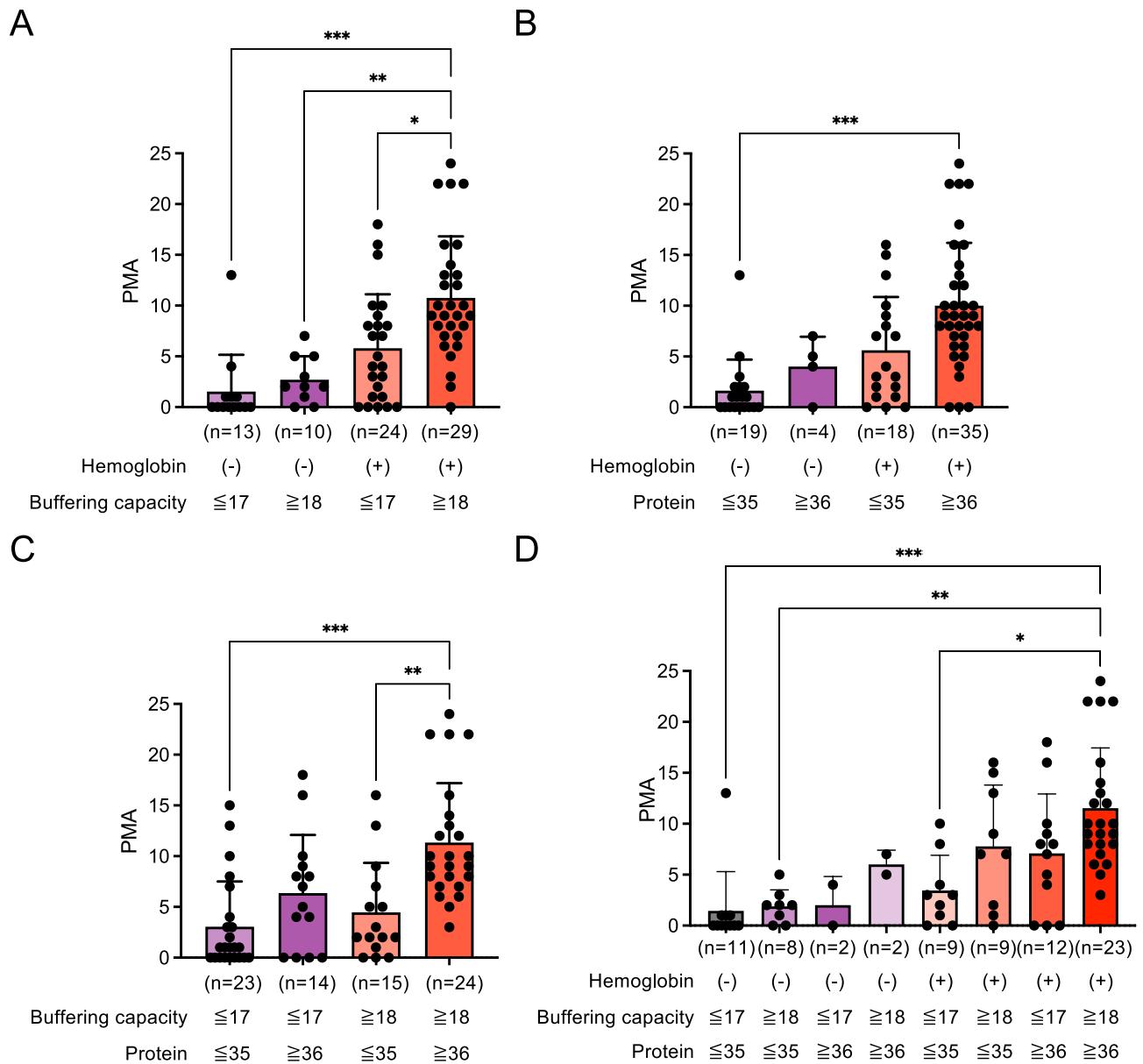


Fig. 5. Relationship between papillary marginal attached (PMA) value, the Perioscreen hemoglobin concentration, and Salivary Multi Test (SMT) items of buffering capacity and protein. (A) Relationship between PMA value, hemoglobin concentration, and buffering capacity. (B) Relationship between PMA value, hemoglobin concentration, and protein. (C) Relationship between PMA value, buffering capacity, and protein. (D) Relationship between PMA value, hemoglobin, buffering capacity, and protein. Each circle represents the data of a subject. Bars represent the mean PMA value. Data expressed as the mean \pm standard deviation. $^*P < 0.05$, $^{**}P < 0.01$, and $^{***}P < 0.001$ between the groups.

buffering capacity and protein using the SMT in addition to conventional hemoglobin testing is effective in detecting subjects at highest risk of periodontal disease.

Because hemoglobin, buffering capacity, and protein each have different sensitivity and specificity, combining them may enable more accurate gingivitis detection. Therefore, we performed a multivariate ROC analysis^{25,26} that combined these indices to examine their accuracy in identifying gingivitis. Among single variables, hemoglobin (AUC = 0.787) demonstrated the highest discrimination performance, followed by protein (AUC = 0.780) and buffering capacity (AUC = 0.744). However, combining variables significantly improved discrimination accuracy, with hemoglobin + buffering capacity (AUC = 0.848), hemoglobin + protein (AUC = 0.834), and buffering capacity + protein (AUC = 0.819) all outperforming the individual models. Furthermore, the model using all three items showed the highest discrimination accuracy (AUC = 0.856), suggesting that it may be a useful predictive model for detecting gingivitis.

Our study has a limitation due to a small sample size of 76 subjects and the fact that there were more males than females. Clinical studies containing essential insights into the diagnosis, risk assessment, and prevention of

No	Explanatory variables	ROC analysis results			Sensitivity	P value (AUC comparison)					
		AUC	95% CI	Cutoff		vs. No. 2	vs. No. 3	vs. No. 4	vs. No. 5	vs. No. 6	vs. No. 7
1	Hemoglobin	0.787	0.683, 0.891	0.5	0.947	0.566	0.897	0.035	0.048	0.579	0.035
2	Buffering capacity	0.744	0.632, 0.855	20.5	0.658	–	0.602	0.045	0.184	0.104	0.032
3	Protein	0.780	0.675, 0.884	35.5	0.763	–	–	0.172	0.134	0.194	0.050
4	Hemoglobin + buffering capacity	0.848	0.762, 0.933	–	–	–	–	–	0.596	0.473	0.577
5	Hemoglobin + protein	0.834	0.743, 0.925	–	–	–	–	–	–	0.706	0.285
6	Buffering capacity + protein	0.819	0.723, 0.915	–	–	–	–	–	–	–	0.222
7	Hemoglobin + buffering capacity + protein	0.856	0.772, 0.940	–	–	–	–	–	–	–	–

Table 3. Multivariate Receiver Operating Characteristic (ROC) analysis using hemoglobin, buffering capacity, and protein as explanatory variables, either singly or in combination, with gingivitis as the outcome measured by papillary marginal attached (PMA) value. AUC; Area under the curve. CI; Confidence interval.

Severe gingivitis

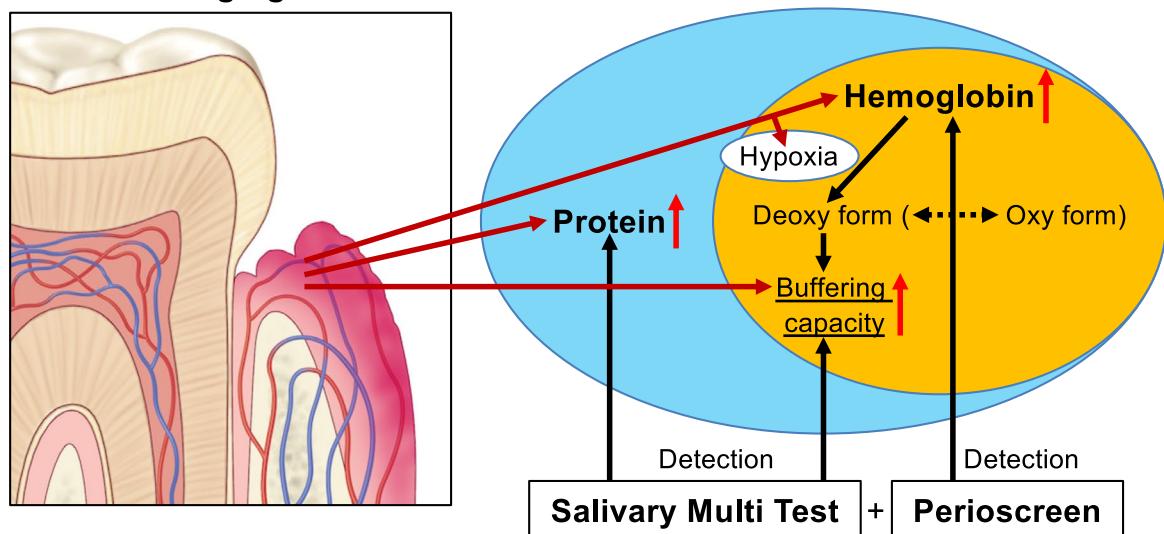


Fig. 6. A possible mechanism for assessing gingivitis risk by the detection of hemoglobin, buffering capacity, and protein from gingival crevicular fluid using the Perioscreen and the Salivary Multi Test (SMT).

oral diseases in pediatric dentistry often involve small sample sizes of several dozen^{27–29}. Meanwhile, systematic reviews and meta-analyses of papers with small sample sizes, ranging from tens to several dozen, have shown promising associations³⁰. Although our study's small sample size limits its statistical power, we believe that our novel diagnostic approach, focusing on hemoglobin, buffering capacity, and protein, may play a helpful role in evaluating pediatric gingivitis. To confirm the findings of this study, further studies with larger sample sizes and increased statistical power are needed.

Another limitation is that we used PMA to evaluate the subjects' gingival inflammation. While PMA can assess the prevalence of gingival inflammation, it is not suitable for determining the severity. To assess the severity of subjects' gingival inflammation, it is preferable to evaluate bleeding on probing, which is recommended by many periodontists as the most reliable method³. In future developments, evaluating bleeding on probing to detect early signs of periodontal disease and prevent its progression is needed.

A possible mechanism for detecting gingivitis risk based on the results of our study is presented in Fig. 6. The Perioscreen detects hemoglobin derived from the gingival crevicular fluid of children with gingivitis. The SMT not only detects proteins including hemoglobin, but also detects the buffering capacity of hemoglobin, especially in the deoxy form. Our findings suggest that a combination of the Perioscreen and the SMT is associated with the prevalence of gingival inflammation and is effective as a novel tool for diagnosing gingival disease in children.

Methods

Subjects

This study was approved by the Hiroshima University Epidemiological Research Ethics Committee (Approval Number: No. E-D56-4). The study was performed in accordance with the Declaration of Helsinki. The subjects were 76 children (50 boys and 26 girls, aged 3–19 years, 8.5 ± 3.9 years (mean age \pm standard deviation), median 7 years) who visited the pediatric dentistry department of Hiroshima University Hospital. Parents and guardians

were informed of the purpose of this study and provided written informed consent. Subjects were recruited consecutively, and those who had received antibiotics within the past month or had abnormalities in the oral mucosa were excluded.

Evaluation of gingival condition

Gingival condition was evaluated by dentists with at least 5 years of experience in pediatric dentistry using the PMA index in accordance with previous studies^{31–33}. Briefly, the labial gingiva between the upper or lower canines (between the primary canines in the case of primary dentition) was divided into three areas: the interdental papilla, the marginal gingiva, and the attached gingiva. Each area was assigned a score of 1 when there was inflammation, and a score of 0 when there was not, and the sum was calculated as the PMA value (the minimum score was 0 and the maximum score was 34).

Perioscreen test

Eating, drinking, and undertaking oral hygiene were not permitted for at least 2 h prior to the test, and the liquid after gargling was collected after rinsing with 3 mL of distilled water for 10 s. The liquid after gargling was dropped onto test paper coated with anti-human hemoglobin monoclonal antibody-conjugated gold colloid provided with the Perioscreen kit³⁴. After 5 min, the color change of the test paper due to the antigen–antibody reaction (0 µg/mL, 2 µg/mL, 5 µg/mL) was judged by comparing it to graded samples.

SMT test

The liquid after gargling was dropped onto a test strip attached to the SMT device and analyzed for 5 min to quantify each factor (cariogenic bacteria, acidity, buffering capacity, white blood cells, protein, and ammonia). The minimum and maximum scores for each item were 0 and 100, respectively.

ROC analysis

We divided 76 subjects into two groups: a low PMA score group (0–5; n=38) and a high PMA score group (6–24; n=38). With PMA as the outcome and hemoglobin, buffering capacity, and protein as individual variables, we performed ROC analysis to determine whether each variable helped identify gingivitis. The Youden index method, which maximizes the sum of sensitivity and specificity, was used to determine cutoff values. Decimal cutoff values obtained using the Youden index were rounded up to the nearest integer for clinical use. Sensitivity, specificity, and AUC were calculated for each cutoff value to evaluate the accuracy of identifying gingivitis. In addition, multivariate ROC analysis was performed using logistic regression analysis, with PMA values as the outcome and hemoglobin, buffering capacity, and protein measurements as explanatory variables. The seven variables analyzed were: (1) hemoglobin alone, (2) buffering capacity alone, (3) protein alone, (4) hemoglobin + buffering capacity, (5) hemoglobin + protein, (6) buffering capacity + protein, and (7) hemoglobin + buffering capacity + protein. ROC curves were created using the predicted values (linear predictors) in each logistic regression model, and the AUC was calculated.

Statistical analysis

Statistical analyses were conducted using GraphPad Prism 9 (GraphPad Software Inc., La Jolla, CA, USA). The results were presented as mean±standard deviation or mean±standard error. Statistical significance was determined using the Kruskal–Wallis test for nonparametric analysis, followed by the Dunn test for multiple comparisons. The correlation between PMA value and the scores of each SMT item was calculated using simple linear regression to obtain the intercept, slope, R squared value, and P value. The test for linear trend was performed using one-way ANOVA. ROC analysis was performed using SPSS for Windows version 22.0 (IBM Japan, Tokyo, Japan), and AUC comparison was performed using R version 4.3.1 (pROC package). Statistically significant differences were determined at $P<0.05$.

Data availability

The data are available from the corresponding author upon reasonable request.

Received: 18 June 2025; Accepted: 16 October 2025

Published online: 20 November 2025

References

1. Research, Science and Therapy Committee of the American Academy of Periodontology. Position paper: periodontal diseases of children and adolescents. *J. Periodontol.* **74**(11), 1696–1704. <https://doi.org/10.1902/jop.2003.74.11.1696> (2003).
2. Oh, T. J., Eber, R. & Wang, H. L. Periodontal diseases in the child and adolescent. *J Clin Periodontol* **29**, 400–410. <https://doi.org/10.1034/j.1600-051x.2002.290504.x> (2002).
3. Lang, N. P. & Bartold, P. M. Periodontal health. *J Periodontol* **89**(Suppl 1), S9–S16. <https://doi.org/10.1002/JPER.16-0517> (2018).
4. Malamud, D. Saliva as a diagnostic fluid. *Dent Clin North Am* **55**, 159–178. <https://doi.org/10.1016/j.cden.2010.08.004> (2011).
5. Slavkin, H. C., Fox, C. H. & Meyer, D. M. Salivary diagnostics and its impact in dentistry, research, education, and the professional community. *Adv Dent Res* **23**, 381–386. <https://doi.org/10.1177/0022034511420435> (2011).
6. Mohanty, P., Gujari, S. K. & Nakum, C. G. Salivary free hemoglobin and lactate dehydrogenase as biomarkers for periodontal disease in smokers. *Quintessence Int* **50**, 428–434. <https://doi.org/10.3290/qi.a42478> (2019).
7. Okada, A. et al. Comparison of salivary hemoglobin measurements for periodontitis screening. *J Oral Sci* **59**, 63–69. <https://doi.org/10.2334/josnusd.16-0204> (2017).
8. Thomas, C. & Lumb, A. B. Physiology of haemoglobin. *Contin. Educ. Anaesth Crit. Care Pain* **12**, 251–256. <https://doi.org/10.1093/bjaceaccp/mks025> (2012).
9. Irie, Y. et al. Investigation of pediatric specifications for the Salivary Multi Test saliva test system. *Pediatr Dent J* **28**, 148–153 (2018).

10. Iwamoto, Y. et al. Application of the salivary multi test in children with severe congenital neutropenia: A case report. *Pediatr Dent J* **33**, 152–158. <https://doi.org/10.1016/j.pdj.2023.05.002> (2023).
11. Ito, H. et al. Usefulness of hemoglobin examination in gingival crevicular fluid during supportive periodontal therapy to diagnose the pre-symptomatic state in periodontal disease. *Clin Oral Invest* **25**, 487–495. <https://doi.org/10.1007/s00784-020-03396-0> (2021).
12. Ahmadi, P. et al. Impacts of *Porphyromonas gingivalis* periodontitis on rheumatoid arthritis autoimmunity. *Int. Immunopharmacol.* **118**, 109936. <https://doi.org/10.1016/j.intimp.2023.109936> (2023).
13. Graves, D. T. & Milovanova, T. Mucosal immunity and the FOXO1 transcription factors. *Front. Immunol.* **10**, 2530. <https://doi.org/10.3389/fimmu.2019.02530> (2019).
14. Jiang, Q. S. et al. Interactions between neutrophils and periodontal pathogens in late-onset periodontitis. *Front. Cell Infect. Mi.* **11**, 627328. <https://doi.org/10.3389/fcimb.2021.627328> (2021).
15. Pandey, R., Gupta, N., Jha, T. & Manzoor, T. B. E. Association of matrix metalloproteinases (MMPs) gene polymorphisms with periodontitis: a systematic review. *GMS Hyg. Infect. Control.* **19**, Doc53. <https://doi.org/10.3205/dgkh000508> (2024).
16. Asikainen, S. & Chen, C. Oral ecology and person-to-person transmission of *Actinobacillus actinomycetemcomitans* and *Porphyromonas gingivalis*. *Periodontol* **2000**(20), 65–81. <https://doi.org/10.1111/j.1600-0757.1999.tb00158.x> (1999).
17. Marchetti, E. et al. Periodontology Part 4: Periodontal disease in children and adolescents. *Eur J Paediatr Dent* **23**, 332–335. <https://doi.org/10.23804/ejpd.2022.23.04.14> (2022).
18. Modeer, T. & Wondimu, B. Periodontal diseases in children and adolescents. *Dent. Clin. North Am.* **44**, 633–658 (2000).
19. Adonogianaki, E., Mooney, J. & Kinane, D. F. Detection of stable and active periodontitis sites by clinical assessment and gingival crevicular acute-phase protein levels. *J. Periodontal Res.* **31**, 135–143. <https://doi.org/10.1111/j.1600-0765.1996.tb00475.x> (1996).
20. Bostancı, N. & Belibasakis, G. N. Gingival crevicular fluid and its immune mediators in the proteomic era. *Periodontol.* **2000**(76), 68–84. <https://doi.org/10.1111/prd.12154> (2018).
21. Preianò, M., Savino, R., Villega, C., Pelaia, C. & Terracciano, R. Gingival crevicular fluid peptidome profiling in healthy and in periodontal diseases. *Int. J. Mol. Sci.* **21**, 5270. <https://doi.org/10.3390/ijms21155270> (2020).
22. Gell, D. A. Structure and function of haemoglobins. *Blood Cells Mol. Dis.* **70**, 13–42. <https://doi.org/10.1016/j.bcmd.2017.10.006> (2018).
23. Linden, A. Measuring diagnostic and predictive accuracy in disease management: an introduction to receiver operating characteristic (ROC) analysis. *J. Eval. Clin. Pract.* **12**, 132–139. <https://doi.org/10.1111/j.1365-2753.2005.00598.x> (2006).
24. Obuchowski, N. A. & Bullen, J. A. Receiver operating characteristic (ROC) curves: review of methods with applications in diagnostic medicine. *Phys. Med. Biol.* **63**, 0701. <https://doi.org/10.1088/1361-6560/aab4b1> (2018).
25. Hu, J., Du, X., Jiang, Y., Wang, Y. & Yang, L. Incorporation of clinical features into a multivariate logistic regression model for the differential diagnosis of benign and malignant TI-RADS 4 thyroid nodules. *Front. Endocrinol. (Lausanne)* **16**, 1550034. <https://doi.org/10.3389/fendo.2025.1550034> (2025).
26. Huang, Y., Pepe, M. S. & Feng, Z. Logistic regression analysis with standardized markers. *Ann Appl Stat* **7**, 1214. <https://doi.org/10.1214/13-AOAS634SUPP> (2013).
27. Han, S. Y. et al. A narrative review on advancing pediatric oral health: comprehensive strategies for the prevention and management of dental challenges in children. *Children (Basel)* **12**, 286. <https://doi.org/10.3390/children12030286> (2025).
28. Mejare, I. A. et al. A systematic map of systematic reviews in pediatric dentistry—what do we really know?. *PLoS ONE* **10**, e0117537. <https://doi.org/10.1371/journal.pone.0117537> (2015).
29. Mitsuhashi, C., Kado, N., Hamada, M., Nomura, R. & Kozai, K. Characterization of the unique oral microbiome of children with Down syndrome. *Sci. Rep.* **12**, 14150. <https://doi.org/10.1038/s41598-022-18409-z> (2022).
30. Mascitti, M. et al. Beyond head and neck cancer: The relationship between oral microbiota and tumour development in distant organs. *Front Cell Infect. Microbiol.* **9**, 232. <https://doi.org/10.3389/fcimb.2019.00232> (2019).
31. Kametani, M. et al. Evaluation of periodontal status and oral health habits with continual dental support for young patients with hemophilia. *Appl. Sci.-Basel* **14**, 1349. <https://doi.org/10.3390/app14041349> (2024).
32. Massler, M. The P-M-A index for the assessment of gingivitis. *J. Periodontol.* **38**(Suppl), 592–601. https://doi.org/10.1902/jop.1967.38.6_part2.592 (1967).
33. Morinushi, T., Lopatin, D. E. & VanPoperin, N. The relationship between gingivitis and the serum antibodies to the microbiota associated with periodontal disease in children with Down's syndrome. *J. Periodontol.* **68**, 626–631. <https://doi.org/10.1902/jop.1997.68.7.626> (1997).
34. Reed, S. G., Manz, M. C., Snipe, S. M., Ohshima, M. & Wagner, C. L. Feasibility study of a salivary occult blood test to correlate with periodontal measures as indicators of periodontal inflammation in a population of pregnant women. *J. Oral Sci.* **57**, 55–58. <https://doi.org/10.2334/josnusd.57.55> (2015).

Acknowledgements

The authors thank Ms. Asami Aoya (Department of Pediatric Dentistry, Graduate School of Biomedical and Health Sciences, Hiroshima University) for her assistance with manuscript editing.

Author contributions

Y. Iwamoto and CM designed the entire study under the supervision of KK and RN. Y. Iwamoto, CM, SI, Y. Ito, SH, ET, YT, AK, MO, TN, MK, SK, YA, and TA collected clinical data. Y. Iwamoto, CM, and RN performed the analyses. Y. Iwamoto, CM, and RN interpreted the data. Y. Iwamoto, CM, and RN wrote the manuscript.

Funding

This study was supported by a grant from Hiroshima University.

Declarations

Competing interests

The authors declare no competing interests.

Additional information

Correspondence and requests for materials should be addressed to R.N.

Reprints and permissions information is available at www.nature.com/reprints.

Publisher's note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Open Access This article is licensed under a Creative Commons Attribution-NonCommercial-NoDerivatives 4.0 International License, which permits any non-commercial use, sharing, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons licence, and indicate if you modified the licensed material. You do not have permission under this licence to share adapted material derived from this article or parts of it. The images or other third party material in this article are included in the article's Creative Commons licence, unless indicated otherwise in a credit line to the material. If material is not included in the article's Creative Commons licence and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this licence, visit <http://creativecommons.org/licenses/by-nc-nd/4.0/>.

© The Author(s) 2025