Prem K. Sreenivasan[1,2,3]
Bhojraj Nandlal[4]
Ganganna Aruna[5]
S. Madhunapantula[6]
Andrew J. McBain[7]

[1] HITLAB, 3960 Broadway, New York, NY 10032, USA
[2] Department of Oral Biology, Rutgers School of Dental Medicine, Newark, NJ. 07103, USA
[3] Adjunct Faculty, JSS Academy of Higher Education and Research, Mysuru, Karnataka State, 570 015, India
[4] Department of Pediatric & Preventive Dentistry, JSS Dental College and Hospital, JSS Academy of Higher Education and Research, Mysuru, Karnataka State, 570 015, India
[5] Department of Periodontics, JSS Dental College and Hospital, JSS Academy of Higher Education and Research, Mysuru, Karnataka State, 570 015, India
[6] Center of Excellence in Molecular biology and Regenerative Medicine (CEMR) Laboratory, Department of Biochemistry JSS Medical College, JSS Academy of Higher Education and Research, Mysuru, Karnataka State, 570 015, India
[7] School of Health Sciences, Faculty of Biology, Medicine and Health, Stopford Building, The University of Manchester, Oxford Road, Manchester, M13 9PT, UK

CORRESPONDENCE
Prem K. Sreenivasan
HITLAB
3960 Broadway
New York
NY 10032
USA
E-mail: Prem.K.Sreenivasan@gmail.com

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The Utility of Salivary Heme to Stratify Healthy Volunteers from Individuals with Gingivitis and Periodontitis

A Pilot Study

KEYWORDS
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Oral
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SUMMARY
Gingival bleeding due to poor oral hygiene is reported globally. Assessment of blood in saliva may improve diagnostics, serve as an outcome measure in clinical trials and support patient education through point-of-care tests. This work analyzed salivary heme using a rapid test format and separately using a lateral flow immunoassay assay (LF) for chair-side implementation. Clinical examinations stratified adult subjects into healthy, gingivitis or periodontitis groups at baseline. Healthy subjects presented no periodontal pockets and whole mouth gingivitis scores of less than 1.0. Gingivitis subjects registered gingival index scores greater than 1.0. Included in the periodontal disease group were subjects with periodontal pockets greater than 4 mm. The rapid test is based on the peroxidase activity salivary heme converting a colorless probe to a colored compound for spectrophotometric analysis. For the LF assay, saliva was placed in the test window of the device with reactions scored after room temperature incubation. Average salivary heme concentrations in the healthy, gingivitis and periodontal disease groups were 27, 201 and 326 nM, respectively, by the rapid test, representing significant differences by analysis of variance and Tukey's-multiple comparison tests ($p < 0.05$). Similarly, results in the LF assay demonstrated increasing band intensity from the healthy to the periodontal disease groups and was quantifiable by image analysis. This pilot study emphasizes the potential efficacy of rapid heme measurement in investigations of oral health.

Introduction

Dental health problems due to inadequate oral hygiene afflict large sections of the population (Petersen & Ogawa 2012). Whereas toothbrushing with a fluoride dentifrice represents important self-care measures (Lindenmuller & Lambrecht 2011; TARTAGLIA et al. 2017), surveys identify the influence of several variables on oral hygiene (Barouch et al. 2019; Broadbent et al. 2011). For example, large amounts of dental plaque approximating 40% of the regions adjacent to the gingival margin are left behind on teeth surfaces after toothbrushing (Petker et al. 2019). Dental plaque comprises large numbers of organisms (Mosaddad et al. 2019) including both Gram-positive and Gram-negative bacteria with generally significantly higher cell density resident on the posterior areas of teeth (Sreenivasan et al. 2016). Microbial growth and metabolism produces products that include acids, toxins, and components with immunogenic features (Takahashi 2015). Features of effective cleaning include the removal of organisms found within each of the distinct oral niches comprising mucosal surfaces, reducing the inflammatory burden (Sreenivasan & Prasad 2020) and improving mucosal integrity (Sreenivasan et al. 2021).

The transition from health to gingivitis and periodontal disease is marked with changes in the inflammatory status of the mouth (Korte & Kinney 2016; Kumar 2019; Lindenmuller & Lambrecht 2011) with investigations reporting increases in matrix metalloproteinase-8 (MMP-8) and lactoferrin in saliva and oral fluids (Ramenzoni et al. 2021a) that correlate with clinical evaluations. An additional aspect of these inflammatory transitions is the presence of trace amounts of blood in saliva (Hofman 2001; Nam et al. 2015; Nomura et al. 2018; Okada et al. 2017). Visualization of blood in saliva has been used to assess the oral health status of patients, in forensic investigations (Chojnowska et al. 2018; Old et al. 2009; Vandenberg & Oorschot 2006), promote oral hygiene (Okada et al. 2017) and examine disease activity (Chojnowska et al. 2018; Hofman 2001; Reed et al. 2015). A variety of analytical approaches including lateral flow immunochromatography, real-time RT-PCR and other rapid approaches (Ji & Choi 2015; Nam et al. 2015; Okada et al. 2017; OLD et al. 2009; Segawa et al. 2019; Vandenberg & Oorschot 2006) are described for the evaluation of saliva, its constituents and heme (Sakurada et al. 2012). Recent clinical investigations have been reported the application of proprietary urinary strips for the non-quantitative assessment of inflammatory markers in saliva including erythrocytes, leukocytes, urobilinogen, nitrite, glucose, bilirubin and ketones (Ramenzoni et al. 2021b). The current evaluation produces a visual outcome for rapidly grading saliva higher concentrations of lactoferrin, hemoglobin and leukocytes being detected in saliva from periodontal disease subjects versus healthy controls.

From a biochemical standpoint, free hemin, resulting from the breakdown of heme-containing proteins such as hemoglobin and myoglobin, differs from heme in that it contains Fe^{3+} instead of Fe^{2+}. Free hemin exists in cells at a very minute concentration (<1µM) and produced due to breakdown of red blood cells or vascular injury and detected in various body fluids such as saliva (Nam et al. 2015; Nomura et al. 2018; Reed et al. 2015; Segawa et al. 2019; Shimazaki et al. 2011), nasal secretions (Sakurada et al. 2012), urine (Linder et al. 2018) and CSF (Lee et al. 2018) under pathological conditions. Furthermore, hemin stimulates the growth of bacteria associated with gingivitis (Smalley & Olczak 2017).

The current investigation determined whether a quantitative test for salivary heme could distinguish patients presenting good oral health from those with gingivitis or periodontal disease as the study hypothesis. A separate objective determined performance of a commercially available lateral flow immunoassay assay (LF) for rapid chairside visual assessment of salivary heme as an adjunct for patient oral health education.

Materials and methods

This double-blind study enrolled adult subjects after the study protocol was approved by the ethics board of the JSS Dental College and Hospital, Mysuru, India (IEC JSS/DCH/Ethical/01/2016-179[2]). All study-related procedures were conducted at the dental clinic of the Dental College with sample analysis conducted in the Center of Excellence in Molecular Biology and Regenerative Medicine (a DST-FIST sponsored center), Department of Biochemistry (a DST-FIST sponsored department), JSS Medical College, Mysuru, India.

Subjects (between the age of 18 and 70 years) who voluntarily completed informed consent were invited to enroll in the study. A dentist interviewed the subjects for their medical and dental history recording demographic variables and completed an oral examination. Study enrollment was restricted to those in good overall health and who were not presently undergoing treatment by a health care professional. Subjects reporting medical or dental procedures or treatments by a health care provider in the month preceding the screening visit were excluded. Also excluded were those scheduled for upcoming treatments or procedures and reporting clinical study participation in the previous month. Subjects reporting a pregnancy or impending pregnancy systemic diseases, infectious diseases or other chronic conditions including diabetes, heart, liver, or kidney were excluded. Study participation was restricted to those presenting more than 20 natural teeth, no oral pathologies, caries, mucosal diseases, dental implants, and restorations. Prospective subjects who required immediate dental care were referred to the specialty clinics of the dental college. Subjects with allergies to oral hygiene formulations or histories of alcohol or drug abuse were excluded.

Clinical evaluations

Enrolled subjects underwent clinical assessments that included an oral soft and hard tissue examination and clinical evaluations for whole-mouth gingival (Löe & Silness 1963) and dental plaque indices (Turesky et al. 1970) along with a periodontal examination (Hefti & Pershaw 2012) and reported no tobacco use. Before subject enrollment, exercises for clinical examiner calibration were conducted for each clinical index utilized in this study. All study related assessments were conducted by the calibrated examiner.

Subject stratification

Enrolled subjects underwent a clinical evaluation during their baseline visit. Based on the outcomes registered during their baseline evaluation, subjects were stratified into the three following groups.

Healthy

Subjects presenting with good oral hygiene, probing depths less than 3 mm, whole-mouth Löe-Silness gingival index scores less than 0.7 and no loss of clinical attachment.

Gingivitis
Subjects who presented with whole-mouth Löe-Silness gingival index scores of 1.2 or more and no loss of clinical attachment.

Periodontal disease
The subjects identified with periodontal disease were determined by clinical criteria. These subjects registered probing depths greater of 4.0 mm or more in at least five sites and clinical attachment loss (CAL) of 4.0 mm or more. Subjects with periodontal disease were also scheduled for bitewing radiographs to examine bone loss (Hefti & Pershaw 2012).

Study enrollment and sampling
After study enrollment, subjects were provided a study schedule for their evaluations. Subjects were instructed to arrive at the dental clinic in the morning prior to oral hygiene and to refrain from food or beverage for two hours prior to their appointments. Saliva samples were collected during these visits. Subject sampling and assessments were completed by 9 a.m.

Saliva sampling
Unstimulated saliva (approximately 2 ml) was collected at each visit representing the baseline, day 8 and day 15 samples. Saliva samples were collected in sterile wide-mouth disposable tubes marked with unique subject identifiers. All collected samples were transported to the laboratory without delay for biochemical analysis. Saliva samples were securely stored under optimal conditions (short term storage: -20 °C; long term storage: -86 °C).

Laboratory analysis

Procedure to detect salivary heme by the colorimetric test
Salivary heme was quantitatively estimated using hemin colorimetric assay kit from BioVision Inc (Catalog #K672-100), Milpitas, CA, USA. The assay relies on the principle that hemin in samples acts as a peroxidase promoting the conversion of a colorless probe to a strongly colored compound, which was measured in a spectrophotometer. Experimentally, first, the probe was warmed at 37 °C for 1–2 mins before use. The enzyme mix was dissolved in hemin assay buffer as is the substrate. All tests included hemin standards (ranging from 0.4 nM to 2.0 nM) prepared using working stock solutions in assay buffer to generate a calibration curve. Controls included reactions without any substrate or missing one reagent. Analysis of the data showed a linear graph with an R^2 value of 0.9916 or higher. The experiment was replicated at least three times with at least two replicate measurements each time.

Saliva samples from subjects were diluted in assay buffer for tests. Diluted saliva samples for assessment were placed in 96-well plates and the volume made up to 50 µl with assay buffer. The reaction mix was added to all wells and incubated in the dark for 30 min before colorimetric evaluation at 570 nm. Heme analyses were conducted in triplicate, and saliva samples diluted appropriately before estimation. In general, samples from healthy subjects were diluted 1:10 and those from gingivitis or periodontal disease diluted 1:100 for testing.

Assessment of heme in saliva using a lateral flow (LF) immunological test
Principle: One-step lateral flow (LF) immunoassay blood test is a commercially available in vitro chromatographic method (Clarity Diagnostics iFOB Rapid Test Cassette, Boca Raton, FL, USA), a CLIA waived test to detect fecal blood, which is not detected by visual observation. This LF chromatographic immunoassay works on the sandwich ELISA principle. The LF assay utilizes a monoclonal antibody to detect human heme and provides a semi-quantitative visual detection in a few minutes. Experimentally, hemoglobin present in the sample first reacts with the anti-hemoglobin antibody coated on a particle. This hemoglobin-antibody complex migrates chromatographically and reacts with anti-hemoglobin antibody present in the membrane. This sandwich complex generates a colored band indicating the presence of hemoglobin in the sample. The method is very sensitive and detects hemoglobin as low as 50 ng/ml.

Method and interpretation of results: The test cassette was placed on a clean, dry, and flat surface. Saliva was added into the device, and positive results recorded if both the control (C) and test (T) line of the strip in the device developed a color. The color intensity of the band was recorded on a semi-quantitative scale ranging from A to H. Samples that did not produce a reaction in the test (T) but produced a reaction in the control (C) line were identified as negative. Invalid tests produced no reaction in either the control (C) and test (T) lines of the strip in the device.

Statistical analysis
Descriptive statistics summarized demographic characteristics of enrolled subjects computed from each clinical group. Included in these summaries were outcomes from clinical evaluations, i.e., dental plaque, gingivitis, clinical attachment loss and pocket probing depths. Results from salivary heme analysis using the colorimetric test are summarized from each clinical group. An analysis of variance (ANOVA) compared the results from each group with Tukey's multiple comparison tests determining differences between groups. Image analyses of LF immunoassay results were evaluated by chi-square test. Statistical tests of hypotheses were two-sided and statistically significant results reported at $p \leq 0.05$.

Results

The demographics of the study population are presented in Table I and includes their clinical characteristics. The study population comprised 25 females and 20 males with an average age of 31 years. Fifteen subjects were identified in the healthy group that included 10 females and 5 males. The 16 subjects placed in the gingivitis group included 8 females and 8 males. The periodontal disease group comprised 14 subjects with 7 male and 7 female subjects. The healthy group registered an average gingival index score of 0.42 representing the lowest score in comparison to either the gingivitis or periodontal disease groups. Average gingival index scores for subjects placed in the gingivitis and periodontal disease groups were 1.34 and 2.41, respectively. Dental plaque index scores progressively increased from the healthy group. Average dental plaque index scores in the healthy, gingivitis and periodontal disease groups were 1.99, 2.22 and 4.24, respectively. While the healthy group registered no clinical attachment loss, the corresponding scores amongst the gingivitis and periodontal disease groups were 0.03 and 1.3, respectively. Average pocket depth in the healthy group was 1.73 mm. Pocket depths averaged 1.6 and 4.3 mm, respectively, amongst the gingivitis and periodontal disease groups. These clinical results remained consistent between the groups at day 8 and day 15 recall visits (data not shown).

Tab. I Summary of subject demographics and their clinical characteristics

Category	Sex		Age (SD)	Gingival index*	Plaque index*	Clinical attachment loss*	Pocket depth (mm)*
Gender	Male (n = 20)	Female (n = 25)	32.24 (11.22)				
Healthy (n = 15)	5	10	22.93 (3.24)	0.42 (0.09, 0.02)	1.99 (0.82, 0.15)	ND	1.73 (0.25, 0.05)
Gingivitis (n = 16)	8	8	30.58 (11.15)	1.34 (0.51, 0.10)	2.22 (0.63, 0.12)	0.03 (0.08, 0.02)	1.60 (0.25, 0.05)
Periodontitis (n = 14)	7	7	42.94 (6.32)	2.41 (0.35, 0.06)	4.24 (0.45, 0.08)	1.34 (1.07, 0.2)	4.38 (1.04, 0.19)

ND, not detected. *Values in parentheses (SD, SEM)

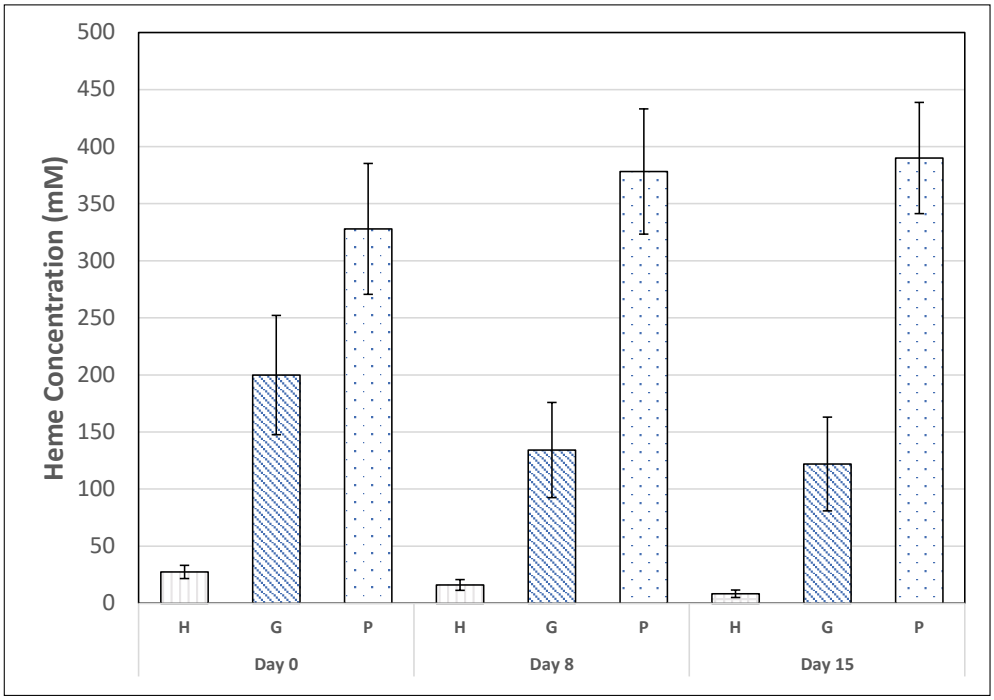

Fig. 1 Salivary heme concentrations over the study period in subjects stratified by oral clinical health status.*
*subject groups represented as healthy (H), gingivitis (G) and periodontal disease (P) demonstrate statistically significant differences.

Analysis of salivary heme by the colorimetric method (Fig. 1) indicates the lowest average heme concentration in the healthy group at baseline and all recall visits with 27 mM recorded at baseline and 16.19 and 8.49 on day 8 and day 15 evaluations, respectively. Average baseline heme concentration amongst the gingivitis group was 200 mM with heme concentrations of 134.3 and 122.1 mM at the day 8 and day 15 evaluations, respectively. Salivary heme concentration in the periodontal disease group was the highest with an average value of 328 nM at baseline. The average values on day 8 and day 15 were 378.2 and 390.0 nM, respectively. Statistical analysis by ANOVA demonstrated significant differences in heme concentration between treatment groups ($p < 0.05$).

Representative images from the lateral flow analysis of heme from saliva samples are shown in Figure 2. Saliva samples from healthy subjects produced the lightest band in comparison to samples obtained from gingivitis or periodontal disease patients. Samples from the gingivitis group produced a band that had a density intermediate to the healthy and periodontal disease groups. Samples obtained from subjects placed in the periodontal disease group produced the strongest reaction with the most prominent bands. A visual semi-quantitative assessment of these bands using a numerical scale ranging from A to H is presented in Table II and includes the results from the entire

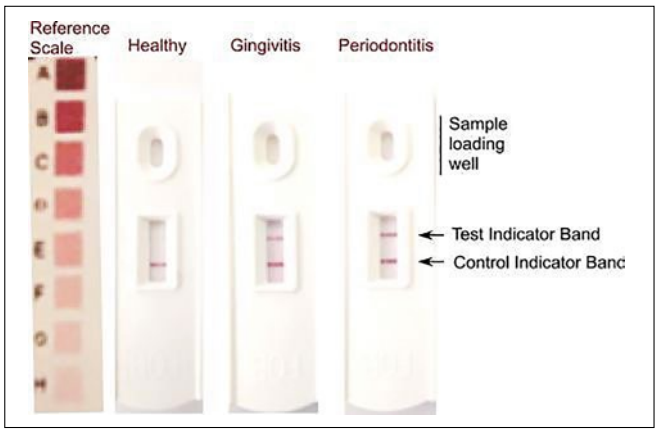

Fig. 2 Analysis of salivary samples by lateral flow assay and representative image.

Tab. II Semi-quantitative visual assessment of color density in lateral flow assay

Score#		Healthy* (n=15)	Gingivitis* (n=16)	Periodontitis* (n=14)
A	8	0.00	0	7.1
B	7	0.00	12.50	57.1
C	6	0.00	25.00	7.1
D	5	0.00	43.75	7.1
E	4	6.67	12.50	21.4
F	3	6.67	6.25	0
G	2	33.33	0	0
H	1	53.33	0	0

Data are percentages #Color intensity of the band recorded on a semi-quantitative scale ranging from A to H. *Data represents the percentage of the responding population.

population. Notably, the healthy group had the highest frequency of bands with the least density with ~80% of the population recording scores in the 1–2 range and corresponded with the G and H letter scores. Samples from healthy subjects had no reactions that were darker than a score of 4, corresponding to the E letter score. The results from the gingivitis and periodontal disease groups were quite different from the healthy group. Both the gingivitis and periodontal disease groups had no reactions that corresponded to the lightly colored bands seen with the healthy group. Gingivitis samples produced bands that clustered in the mid-portion of color density. The gingivitis group recorded the highest frequency of results with a score of 5 corresponding to the D letter score. In contrast, the periodontal disease samples produced the darkest bands. Results from this group clustered toward the darker regions of the semi-quantitative scale. An additional objective measure of these bands by image-J analysis is presented in Figure 3. These results corroborate the semi-quantitative visual readings.

Discussion

Global surveys of oral health and hygiene indicate the widespread prevalence of common oral diseases including gingivitis and periodontal disease (Kumar 2019; Lertpimonchai et al. 2017; Petersen & Ogawa 2012; Lindenmuller & Lambrecht 2011). Whereas a significant focus is on biomarkers and emerging technologies in examining the transition from health to oral disease (Korte & Kinney 2016; Kumar 2019; Lindenmuller & Lambrecht 2011), we assessed a relatively rapid approach along with a rapid chair-side test to detect salivary heme under resource-restricted conditions. The advantages of these efforts include rapid screening of populations without extensive technical training with the tests conducted under ambient conditions following room temperature storage of reagents.

This pilot study included well-described clinical indices widely used to identify the oral health status of subjects (Hefti & Pershaw 2012). Clinical indices are widely accepted gold standard to determine oral health status but require a trained dentist and have some identified drawbacks such as (a) patient discomfort; (b) the use of invasive approaches such as probing, and (c) minimal flexibility for large scale screening of populations. In the present study subjects were stratified by widely accepted clinical criteria (Löe & Silness 1963; Turesky et al. 1970; Hefti & Pershaw 2012). The tests evaluated in this investigation provide quantitative and visual outcomes that can be interpreted easily or photographed for tele dentistry or longitudinal monitoring.

Biomarkers offer important advantages and new avenues to assess oral health status (Ramenzoni et al. 2021a, 2021b; Ji & Choi 2015; Korte & Kinney 2016) and systemic diseases (Hofman 2001; Song et al. 2018). Saliva representing a biological sample is widely recognized for its significant advantages due to its relative ease of collection, sampling flexibility, transportation and biobanking (Ramenzoni et al. 2021a, 2021b; Ngamchuea et al. 2017). Available in the literature are reports evaluating saliva for heavy metals, hormones, and enzymes (Old et al. 2009). Saliva with its analytes has a broad appeal as an important diagnostic specimen for antibodies, drugs, hormones, and applications in forensic investigations (Chojnowska et al. 2018; Hofman 2001; Vandenberg & Oorschot 2006). Automated biochemical test for-

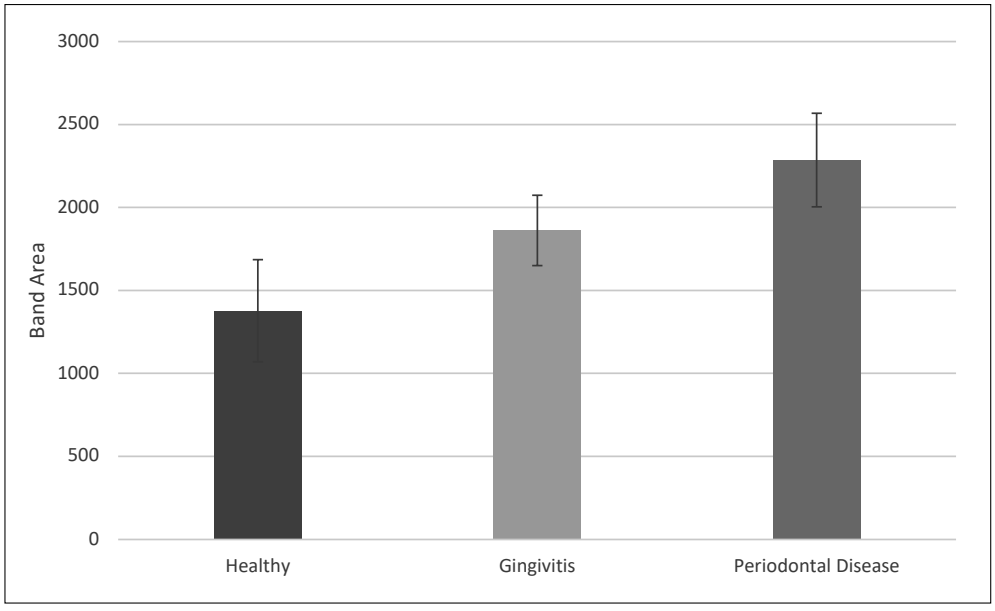

Fig. 3 Quantification of lateral flow immunoassay results by image analysis.

mats with the collected saliva will further advance the design of longitudinal studies evaluating interventional strategies.

An important aspect of the present study were standardized procedures with sample collection and assessments conducted in the morning prior to oral hygiene. Clinical examinations were conducted by a calibrated examiner and laboratory assessments conducted in triplicate with appropriate controls to ensure replication and robust statistical analyses. Using these approaches, statistically significant differences were noted between the groups. The rapid assay for salivary heme can be readily conducted with small amounts of sample for quantitative outcomes. Consequently, it is applicable for a variety of patients, clinically difficult situations and circumstances that restrict sample availability.

The literature includes many reports on salivary heme. The Japanese pharmaceutical affairs law allows screening for periodontal disease using salivary hemoglobin (Okada et al. 2017). In Japan, a few salivary hemoglobin tests are approved as in vitro diagnostics. Rapid diagnostic test formats including latex agglutination and others modified from fecal occult blood tests are reported (Okada et al. 2017). A commercially available LF test using monoclonal antibody for human heme detection in fecal samples was evaluated in this investigation. Outcomes from the LF assay corroborated the results from the rapid heme test distinguishing the three clinical groups readily in the recall visits over the study period. Prominently, subjects in health consistently demonstrated lower heme scores than those with gingivitis or periodontal disease reflecting clinically relevant outcomes. A substantial overlap in heme results was noted between the gingivitis and periodontal disease groups. Likely reasons for these observations include the inability of clinical scores to adequately stratify subjects as either gingivitis or periodontal disease and the episodic nature of heme amongst diseased subjects (Okada et al. 2017). The ability to identify healthy subjects represents important outcomes of relevance and can aid patient engagement efforts. Furthermore, saliva storage for subsequent testing provides greater flexibility for analysis with the evaluations supplementing the well-accepted clinical indices (Löe & Silness 1963; Turesky et al. 1970; Hefti & Pershaw 2012).

In summary, results from this study demonstrate the utility of a rapid quantitative method to detect heme in human saliva. Potential limitations of this study include its smaller sample size but its advantages such as evaluation of small volumes, sample storage and other flexibilities to augment the design of oral health surveys or clinical studies examining interventions require highlight. The LF assay is readily adaptable and can be conducted under ambient conditions supporting asynchronous at-home sampling and reporting capabilities. Importantly, it can be conducted in remote regions with limited access to clinical or laboratory facilities. While it does not require extensive technical training to be conducted, it facilitates patient educational, maintenance of quantitative longitudinal records to augment teledentistry including remote monitoring. The LF test format is available for many other analytes and widely used by a variety of health care providers including physicians, nurses, health aids representing a widely adopted technique to monitor health-related outcomes. In this regard, the described LF-based analyses facilitates the delivery of oral care by physicians, nurses and others representing the entire spectrum of health care providers. Consequently, the LF assay also affords the possibility of screening larger populations alleviating circumstances constrained by time and resources. Future efforts measuring heme as outcome measures are required to evaluate the effects of interventions on augmenting oral health.

Zusammenfassung

Einleitung

Zahnfleischbluten aufgrund mangelnder Mundhygiene und eine entsprechende Verschlechterung der oralen Gesundheit sind globale Phänomene. Entzündliche Veränderungen im Mund während der Entstehung von Gingivitis oder Parodontitis werden von spezifischen Veränderungen in zellulären und anderen Biomarkerprofilen im Speichel begleitet. Blutbestandteile im Speichel können Veränderungen in der oralen Gesundheit anzeigen, die zur Patientenaufklärung und auch für Zahnärzte und anderes Gesundheitspersonal interessant sein könnten. Viele klinische Studien haben Blutbestandteile im Speichel untersucht mit dem Ziel, die Diagnostik zu verbessern, die orale Gesundheit über die Zeit zu beobachten, den Ausgang von Behandlungsoptionen darzustellen oder einfach um Patienten mittels Direkt-(Point-of-Care-)Tests aufzuklären. Die vorliegende Studie widmete sich einer quantitativen kolorimetrischen Methode und einem Lateral-Flow-Assay zur Detektion von Häm für die direkte Bestimmung der oralen Gesundheit.

Material und Methoden

Eine klinische Untersuchung wurde durchgeführt, um Patienten in drei Gruppen einzuteilen: gesund (n = 15), Gingivitis (n = 16), Parodontitis (n = 14). Gesunde Subjekte hatten keine Zahnfleischtaschen und Löe-Silness-Scores von weniger als 0,7 ohne Attachmentverlust. Gingivitis-Patienten hatten entsprechende Werte von über 1,2. Parodontitis-Patienten hatten Taschen von über 4 mm an mindestens fünf Stellen. Es wurde zu drei Zeitpunkten unstimulierter Speichel gesammelt, mit jeweils einer Woche Karenzzeit. Die quantitative kolorimetrische Methode braucht eine kleine Menge von Speichel. Sie basiert auf der Peroxidase-Aktivität vom Häm und einer entsprechenden im Assay spektrofotometrisch messbaren Farbreaktion. Vorteile dieser Methode sind rasche Resultate und die Möglichkeit, viele Tests auf einmal durchzuführen. Für das Lateral-Flow-Assay wurde der Speichel ins entsprechende Fenster des Tests gegeben (entsprechend einem Covid-Test), und das Resultat wurde einige Minuten nach Inkubation bei Raumtemperatur abgelesen. Dieses Assay hat den Vorteil, dass es keine zusätzlichen Apparaturen braucht und auch von Patienten zu Hause selbst durchgeführt werden könnte. Das Resultat könnte man fotografieren und zur Kontrolle beziehungsweise Quantifizierung mittels einer Bildanalyse-Software verwenden.

Resultate

Die durchschnittliche Hämkonzentration im Speichel, die mittels kolorimetrischen Tests bestimmt wurde, war 27 nM bei den gesunden, 201 nM bei den Gingivitis- und 326 nM bei den Parodontitis-Patienten. Diese Unterschiede waren statistisch signifikant ($p < 0{,}05$). Die Hämwerte im Speichel blieben über die Zeit konstant. Auch die Resultate des Lateral-Flow-Assays zeigten steigende Bandenintensität von gesund bis Parodontitis und waren mittels Bildanalyseverfahren quantifizierbar.

Diskussion

Mittels der hier beschriebenen Methoden wurden unterschiedliche Hämwerte im Speichel von gesunden Patienten und solchen mit Gingivitis oder Parodontitis gemessen. Diese

Methoden könnten nützlich sein zur Patientenmotivation am Behandlungsstuhl. Die hier vorgestellten Methoden könnten zudem zum Selbstmonitoring zu Hause, zum schnellen Screening einer grösseren Population und zur Anwendung durch Ärzte oder anderes Gesundheitspersonal verwendet werden, und zwar ohne Einschränkungen durch Zeit und andere allenfalls nicht vorhandene Ressourcen.

Résumé

Introduction

Les saignements de gencive dus à une hygiène buccale insuffisante et la détérioration correspondante de la santé orale sont des phénomènes globaux. Les changements inflammatoires dans la bouche au cours de l'apparition d'une gingivite ou d'une parodontite s'accompagnent de modifications spécifiques des profils cellulaires et autres marqueurs biologiques dans la salive. Les composants sanguins présents dans la salive peuvent indiquer des changements relatifs à la santé bucco-dentaire, ce qui pourrait être intéressant pour l'information aux patients et également pour les médecins-dentistes et les autres professionnels de la santé. De nombreuses études cliniques ont investigué les composants sanguins dans la salive dans le but d'améliorer le diagnostic, de suivre l'évolution de la santé orale sur la durée, de montrer les résultats des options thérapeutiques choisies ou tout simplement de renseigner les patients au moyen de tests directs *(point-of-care)*. La présente étude a été consacrée à une méthode colorimétrique quantitative et à un test à flux latéral *(Lateral-Flow-Assay)* pour la détection de l'hème afin d'évaluer directement la santé orale.

Matériel et méthodes

Un examen clinique a été réalisé afin de répartir les patients en trois groupes : bonne santé orale (n = 15), gingivite (n = 16), parodontite (n = 14). Les participants en bonne santé orale n'avaient pas de poches gingivales et un *score de Löe-Silness* inférieur à 0,7 sans perte d'attache. Le score correspondant des patients présentant une gingivite était supérieur à 1,2. Les patients présentant une parodontite avaient des poches de plus de 4 mm dans cinq localisations au moins. De la salive non stimulée a été collectée à trois moments, à intervalles d'une semaine. La méthode colorimétrique quantitative nécessite une petite quantité de salive. Elle est basée sur l'activité peroxydasique de l'hème et sur la réaction colorimétrique correspondante mesurable par spectrophotométrie lors du test. Les avantages de cette méthode sont les suivants : des résultats rapides et la possibilité d'effectuer de nombreux tests en une seule fois. Pour le test à flux latéral, la salive était placée dans la fenêtre correspondante du test (correspondant à un test COVID) et le résultat était lu après quelques minutes d'incubation à température ambiante. Ce test a l'avantage de ne pas nécessiter d'appareillage supplémentaire, et pourrait aussi être réalisé par les patients à domicile. Le résultat pourrait être photographié et utilisé comme contrôle ou pour la quantification au moyen d'un logiciel d'analyse d'images.

Résultats

La concentration de l'hème dans la salive, déterminée par test colorimétrique, a été en moyenne de 27 nM chez les patients en bonne santé orale, de 201 nM chez les patients présentant une gingivite et de 326 nM chez les patients présentant une parodontite. Ces différences étaient statistiquement significatives ($p < 0{,}05$). Les taux de l'hème dans la salive sont restés constants au cours de la période d'analyse. Les résultats du test à flux latéral ont également montré une intensité croissante des bandes – à partir des patients sains jusqu'aux patients présentant une parodontite –, et ces résultats ont pu être quantifiés à l'aide d'une méthode d'analyse d'images.

Discussion

Les méthodes décrites ici ont permis de mesurer des valeurs de l'hème différentes dans la salive des patients en bonne santé orale par rapport aux patients présentant une gingivite ou une parodontite. Ces méthodes pourraient être utiles pour la motivation des patients au fauteuil. Les méthodes présentées ici pourraient en outre être utilisées pour l'autosurveillance à domicile, pour le dépistage rapide d'une population assez importante et pour être appliquées par des médecins ou d'autres professionnels de la santé, et cela sans limitation par des contraintes de temps ou par l'absence éventuelle d'autres ressources.

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