Sulcular Sulfide Monitoring: An Indicator of Early Dental Plaque-Induced Gingival Disease

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Purpose. The purpose of this study was to evaluate the relationship between volatile sulfur compounds (VSC) and gingival health status and to determine if volatile sulfur compounds can detect early dental plaque-induced gingival disease.

Methods. A split-mouth design with randomly selected quadrants of the mandibular arch enabled 39 participants to serve as their own controls. At baseline and at three subsequent appointments (days 7, 14, and 21) gingival inflammation (GI), bleeding on probing (BOP), and sulfide levels (SUL) were measured using the Gingival Index and the Diamond Probe/Perio 2000 System. For three weeks, participants refrained from brushing and flossing one randomly selected quadrant of the mandibular arch. The Pearson correlation test was used to determine the relationship between sulfide concentrations and gingival health. The Wilcoxon signed rank test was used to compare the differences in mean GI, BOP, and SUL scores between the hygiene side (H) and the non-hygiene side (NH).

Results. Data suggest that SUL correlate positively to GI and BOP on both sides; however, the strength of the correlation was stronger for the NH side. A comparison of mean GI, BOP, and SUL scores revealed a statistically significant difference between sides for all three parameters from baseline to day 21, except for SUL on day 14.

Conclusions. Based on study outcomes, the Diamond Probe/Perio 2000 System demonstrated the ability to detect sites with elevated SUL; therefore, SUL may be a useful adjunctive indicator of early plaque-induced gingivitis. In addition, data revealed a moderate correlation between SUL levels and gingival inflammation on the NH sides. Whether sulfur by-product is a contributor to the disease process, or merely a correlate, is inconclusive.

Introduction

Periodontal disease includes a group of inflammatory conditions of the periodontal tissues broadly categorized as gingivitis and periodontitis. Inflammation of the gingiva induced by plaque biofilm is the most common form of gingivitis.[1] Experimental research in humans has long confirmed bacterial plaque as a cause of gingival disease.[2] Moreover, the

American Academy of Periodontology supports the theory that the initiation and progression of most forms of gingivitis are dependent upon the presence and persistence of specific pathogens in bacterial plaque.[3] As gram-negative bacteria cross the epithelial barrier into the underlying connective tissue of the periodontium, an inflammatory reaction in the host is initiated, leading to connective tissue breakdown. Gram-negative bacteria have the potential to generate volatile sulfur compounds (VSC), specifically, hydrogen sulfide (H_2S), methyl mercaptan (CH_3SH), and dimethyl sulfide (CH_3SCH_3), as by-products of their metabolism.[4] These by-products of bacterial metabolism may be factors in the etiology and pathogenesis of periodontal disease.[5]

Numerous studies reveal that sulfur by-products are associated with periodontal disease.[6,7,8] Additionally, researchers suggest that even in low concentrations, VSC are highly toxic to periodontal tissue and may contribute to the etiology of gingivitis.[9] Tonzetich proposed that VSC might alter the permeability of affected cells and facilitate the transmission of toxic metabolites into underlying connective tissue, which contributes to the progression of periodontal disease.[10] Yaegaki and Suetaka found that concentrations of VSC precursors increased with the severity of periodontal disease.[5] The ratio of CH_3SH and H_2S significantly increased in patients with periodontal disease in proportion to bleeding and probing depth. The amounts of H_2S and CH_3SH in infected periodontal pockets were considered high; however, the validity of VSC as an indicator of plaque-induced gingivitis and its relationship to oral health is still unknown.

As plaque bacteria and bacterial by-products interact with the host, inflammation and tissue destruction results, leading to the clinical signs and symptoms of gingivitis. Gingivitis is a necessary precursor to the development of periodontitis; however, not all gingivitis progresses to periodontitis.[11] In an attempt to develop objective measures of disease activity, studies have tested saliva, blood, plaque, and gingival crevicular fluid (GCF)[10,12,13,14] Bleeding, a classic sign of inflammation, and periodontal probing have long been used as standard measures of disease activity.[13] Bleeding on probing (BOP) and the Gingival Index (GI) have been used to clinically characterize the degree of clinical gingival inflammation; however, it is becoming increasingly apparent that traditional clinical criteria are inadequate for determining active disease sites, quantitatively monitoring the response to therapy, and/or measuring the degree of susceptibility to future breakdown. For many years, researchers have sought refinements in the sampling of blood, saliva, plaque and GCF. The availability of more analytical techniques for detecting disease-associated bacteria in subgingival plaque gives significant cause for optimism.[6]

Since research suggests a correlation between sulfides and plaque-induced gingivitis, the presence of sulfides in the gingival sulcus should alert the clinician to the possibility of disease activity.[11] The cytotoxicity of H_2S and CH_3SH strongly suggests that VSC may play an etiologic role in the gingival disease process 6 since both H_2S and CH_3SH are capable of inducing a change in the structure of the crevicular epithelium and initiating the destructive inflammatory process.[15] Rizzo indicated that detectable hydrogen sulfide is produced in the deepest portion of periodontal pockets in humans;[16] therefore, detecting the presence or absence of VSC within the periodontal pockets could be a direct measure of the gram-negative bacterial activity.

The Diamond Probe/Perio 2000 System™ is a device that measures sulfide levels as an adjunct to traditional diagnostic techniques in the evaluation of periodontal disease. Standard periodontal probing is a retrospective analysis of disease, whereas the Diamond Probe/Perio 2000 System provides a real-time indicator of sulfide activity. The system reduces uncertainty about the presence of gram-negative bacterial activity in the gingival sulci by detecting various forms of sulfides (S^-, HS^-, H_2S, and CH_3SH). The presence of relative sulfide concentrations in individual sulcus sites alerts the practitioner to the presence of bacterial activity, therefore improving clinical decision-making in periodontal assessment.

Review of the Literature

Researchers have studied VSC as intermediate products from the bacterial putrefaction of proteins with sulfur-containing amino acids and other proteins in the human oral cavity. Studies have demonstrated that H_2S and CH_3SH are predominant

components of VSC.[4,17-19] Additional studies have been conducted to determine whether or not H_2S and CH_3SH gases actually do cause significant tissue destruction in the oral cavity.[9,14,15,19-23]

Research suggests that the presence of SUL in healthy people may be influenced by individual differences, the aging of the plaque in the absence of oral hygiene procedures for 24 hours prior to data collection, and absorption from the diet.[24] This observation is supported by the work of Yaegaki and Sanada, who found that VSC concentrations were higher in mouth air from individuals with BOP than those with no BOP.[14]

Upon observing an increased volume of VSC in the oral cavity of individuals with periodontal disease, Rizzo pointed out that H_2S is one of the most toxic metabolic by-products of anaerobic bacteria located in periodontal pockets.[16] In the oral cavity, the frequency of anaerobic bacteria increases in periodontitis as compared with healthy sites. Langendijk, et al determined that periodontal sulfate-reducing bacteria are associated with several clinical categories of periodontitis and with periodontal sites of increased pocket depth.[24] Toxic sulfide products of these strictly anaerobic bacteria can accumulate in periodontal pockets in concentrations that may cause cellular destruction. Ng and Tonzetich suggested that H_2S adversely affects protein synthesis of human gingival fibroblasts in culture.[15] Upon exposure of collagen to elevated H_2S concentrations, the H_2S converted some acid-soluble collagen to a more soluble product. This effect on collagen solubility makes it more susceptible to enzymatic degradation and contributes to the increased destruction of collagen observed in thoil-treated fibroblast cultures.[7]

Tonzetich and McBride observed differences in intermediate sulfide metabolism between non-pathogenic strains of *Bacteroides melaninogenicus var melaninogenicus* (CP-) and pathogenic *Bacteroides melaninogenicus* (CP+)[25] The CP+ strains, which produced collagenase and protease and caused the formation of abscesses when injected subcutaneously into the groins of guinea pigs, produced copious amounts of VSC, which consisted predominantly of CH_3SH. Although the CP- organisms did not grow as well as the CP+, the differences in concentration of VSC may be only partly related to the disparity in growth rates. Results suggest that VSC analysis offers a convenient means of assessing strain differences and pathogenic potential of *Bacteroides melaninogenicus*. The addition of glucose to the medium depressed total volatile sulphur production by both CP+ and CP- strains, attributable mostly to lower H_2S levels.

Persson et al reported that subgingival microbiotas recovered in deep periodontal pockets had a very high capacity to produce VSC in human serum.[19] Bacterial samples taken from 7mm to 12mm periodontal pockets were incubated for seven days to determine the amount of VSC and the degradation of serum protein produced.[19] Results suggest that H_2S was the predominant VSC in the serum, but significant amounts of CH_3SH also formed. In addition, members of subgingival microbiotas had the capacity to degrade human serum proteins and form VSC compounds.

Persson revealed that the most potent producers of H_2S were *Trepomena denticola* and black-pigmented species, *Bacteriodes intermedius*, *Bacteroides loescheii*, *Porphyromonas endodontalis*, and *Porphyromonas gingivalis*. *Porphyromonas gingivilas* also produced copious amounts of CH_3SH in serum protein,[26] suggesting that VSC initiate and perpetuate an inflammatory response, producing tissue irritation, bleeding, and increase of susceptibility to periodontal breakdown in the future.

Researchers have established a direct correlation between the amounts of H_2S and CH_3SH and the severity of periodontal disease activity.[7,14] By using radioactive, electrophoretic, and chromatographic techniques, Tonzetich and Lo observed the reactions of H_2S within saliva, collagen, and gelatin.[22] The exposure of oral mucosa to both H_2S and CH_3SH caused a marked increase in its permeability. These findings support the involvement of sulfide compounds in the etiology of periodontal disease. Since H_2S has been identified in periodontal pockets and is implicated in periodontal disease, results of the H_2S treatment of collagen and saliva could provide insight into the possible mechanism of involvement in the disease process.

Periodontal pockets are believed to be one of the main production sites of oral VSC. The generation of H_2S and CH_3SH could be due to the presence of the specific gram-negative organisms in gingival crevicular fluid (GCF) or the effect of

enzymes produced by organisms in the fluid. The clinical presentation of gingivitis is important because of the association of elevated sulfide levels and disease;[6] therefore, the adjunctive use of VSC monitoring is promising.
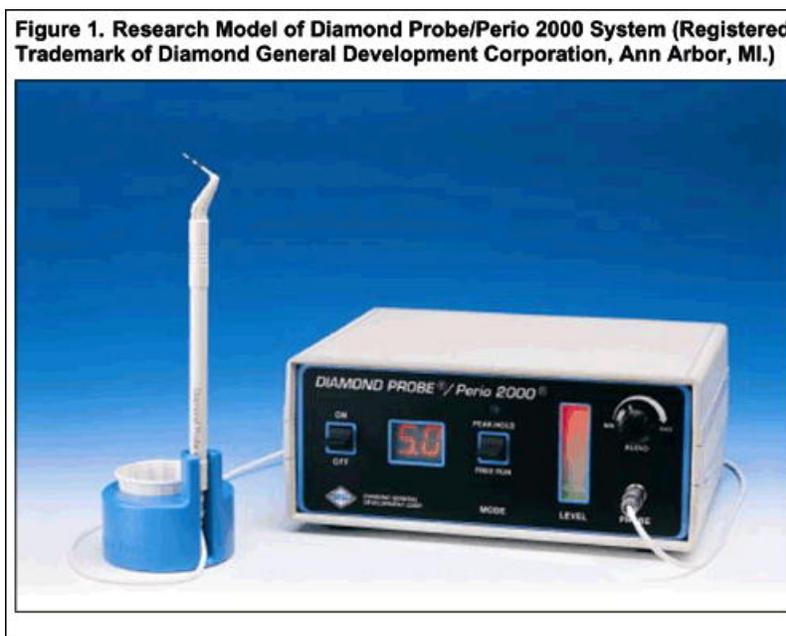
Methods and Materials

Thirty-nine individuals, between ages 19 and 62, who were in good general health, not pregnant, free of orthodontic and prosthetic appliances, had a Gingival Index score of 0 to 1, and were free of antibiotics for one month prior to data collection were enrolled. Among the 39 participants, 28 (71.79%) were women and 11 (28.21%) were men with a mean group age of 25.67 years. The informed consent approved by the Institutional Review Board of Old Dominion University was signed in duplicate, with one copy given to the individual and one copy placed on file. Participants were recruited via flyers distributed throughout the university community and campus wide e-mail system. A single dental hygienist was calibrated for intra-rater reliability prior to data collection.

Each participant's mandibular arch was randomized to hygiene (H) and non-hygiene (NH) sides to allow each individual to serve as his/her own control. At baseline, all participants were given the same type of soft toothbrush and fluoride toothpaste without added ingredients for controlling gingivitis or calculus formation (Crest Regular Sodium Fluoride Toothpaste. Procter and Gamble, Cincinnati, OH). No attempt was made to modify participants' daily oral hygiene except on the NH side where individuals were advised to refrain from oral care for 21 days. Participants were instructed not to use mouth rinse for the duration of the study.
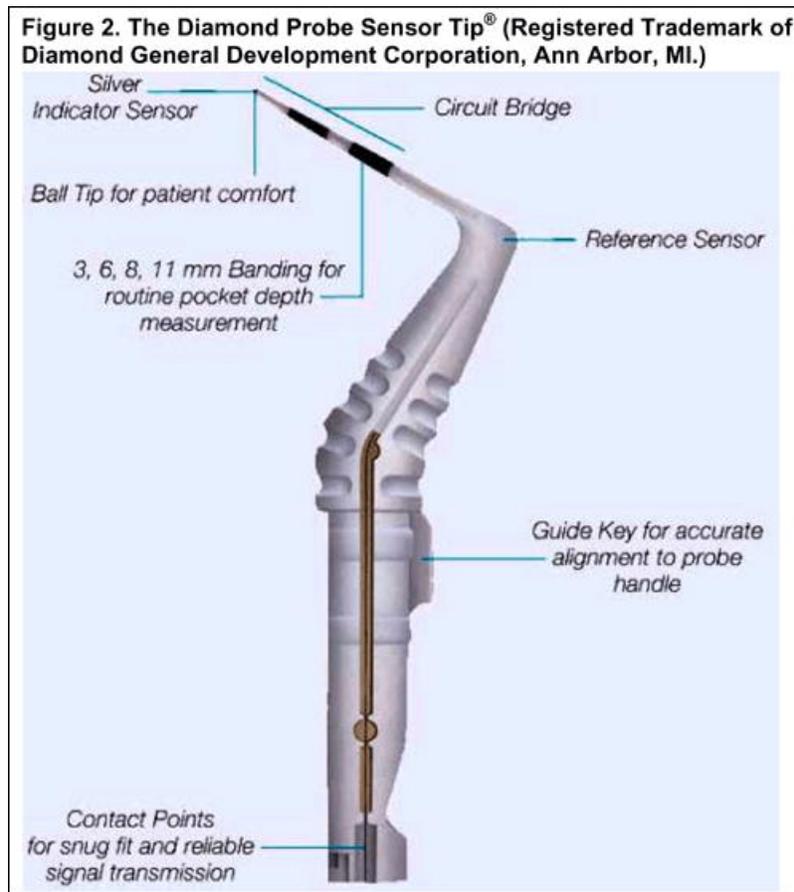
A full mouth (excluding third molars) periodontal probing was performed at four sites (distofacial, facial, mesiofacial, and midlingual) to determine baseline periodontal health status. GI, BOP, and SUL scores were obtained at the same four sites at baseline and three subsequent data collection appointments over a 21-day period (days 7, 14, and 21). At the completion of the study, all participants were offered a prophylaxis and were followed to ensure their return to baseline gingival health status.

The Diamond Probe/Perio 2000 System is a real-time, chairside system designed to evaluate relative gram-negative bacteria in the gingival sulci and periodontal pockets (Figure 1). When sulfides are encountered in the GCF, the system provides information three ways: through a lighted display, by emitting an audible tone, and by providing quantitative sulfide levels.

Figure 1. Research Model of Diamond Probe/Perio 2000 System (Registered Trademark of Diamond General Development Corporation, Ann Arbor, MI.)

Diamond Probe Sensor Tip® incorporates a micro-sulfide sensor into a modified "Michigan O" style disposable periodontal probe to measure probing depth, BOP, while detecting the presence of sulfides (Figure 2). The thin, rounded probe tip has

incremental color-coded markings at 3, 6, 8, and 11 mm for easy measurement. The tip diameter of the Diamond Probe is 0.38 mm, which is the same diameter as the "Michigan O" probe.

Figure 2. The Diamond Probe Sensor Tip® (Registered Trademark of Diamond General Development Corporation, Ann Arbor, MI.)

Various forms of sulfides (S⁻, HS⁻, H2S, and CH3SH) within the GCF interact with the sensor tip of the probe and generate a signal that is converted to visual and audible alerts. The sensor tip is sensitive to one part per million molar sulfide (106 mol. S). The digital format displays a measurement of sulfides ranging from 0 to 10,000 units quantified in increments of 0.5. Additionally, an analog display provides a four-color light bar that scales up and down to correlate with sulfide concentrations-from green (low) to red (very high)-along with an audible tone. The Diamond Probe/Perio 2000 System was used according to the manufacturer's directions.

Each of the four gingival areas (distofacial, facial, mesiofacial, and midlingual) were assigned a GI score from 0 to 3.

0=Normal gingival status

1= Mild inflammation-slight color change, slight edema. No bleeding on probing.

2= Moderate inflammation-redness, edema, and glazing. Bleeding on probing.

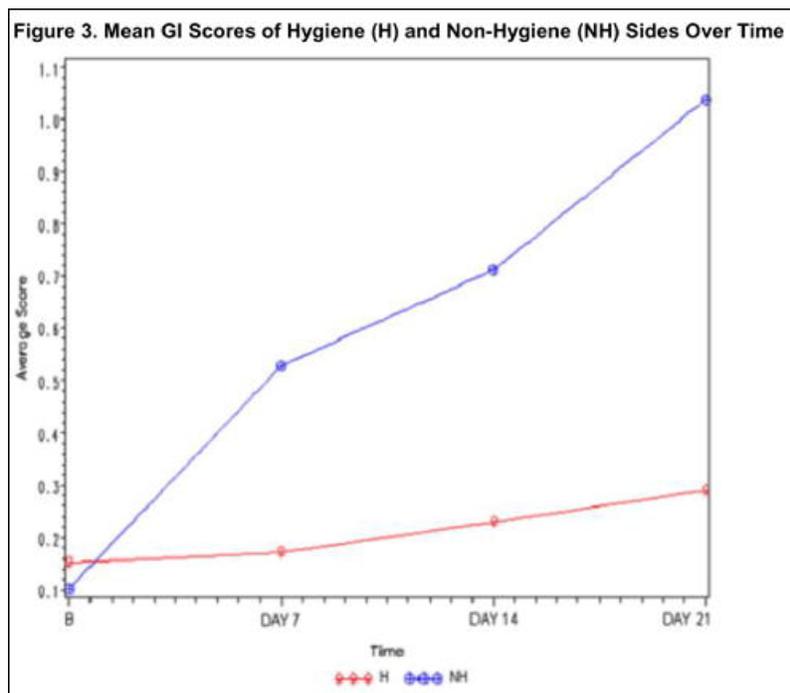3= Severe inflammation-marked redness and edema, ulceration. Spontaneous bleeding.

BOP was additionally recorded as 0 (negative) or 1 (positive)
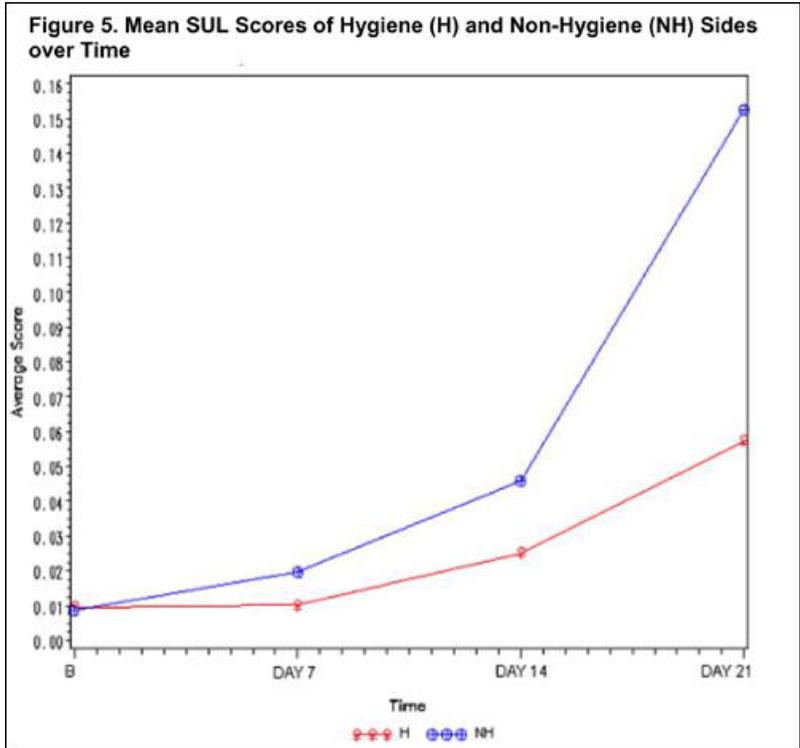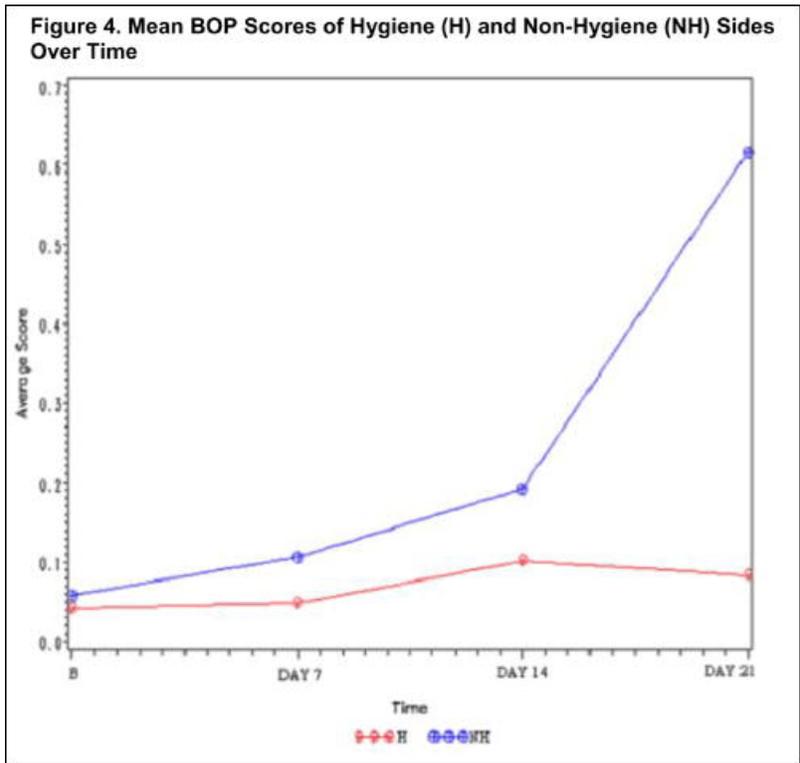
If SUL were detected, the probe tip was removed from the sulcus, washed to remove residual sulfides, and reset for the next measurement. After completing the examination of the lingual or buccal sections of a quadrant, even without SUL or BOP present, the probe tip was re-washed. This procedure was repeated when plaque from the sulcus was visible on the probe tip.

Results

The present study evaluated the relationship of sulfides and early dental plaque-induced gingivitis. Subsequent statistical analyses were conducted on average mean scores using individual teeth as the observation (or data). Data were analyzed using the mean as the most common measure of central tendency, percentiles describing the relative position of a given score in relation to other scores. The Pearson correlation test was used to determine the relationship between SUL, BOP, and GI. The Wilcoxon signed rank test was used to compare the differences in mean scores in GI, BOP, and SUL between H and NH sides. Since no multivariate nonparametric tests were readily available for application, testing was performed for GI, BOP, and SUL separately using the Wilcoxon signed rank statistic at the .05 level. To perform four comparisons for each GI, BOP, and SUL, a Bonferroni method of multiple comparisons will have a component-wise level of significance at the 0.0125 level. Even at the p=.01 significance level, most of the conclusions remain valid.

Overall mean scores of the GI, BOP, and SUL levels are presented in Table I. Visual comparisons of mean scores are presented in Figures 3, 4, and 5. Mean GI, BOP, and SUL scores on the NH side remained higher than on the H side throughout the study. GI scores on the NH side increased significantly from baseline to day 7, and then continued to increase, peaking at day 21; whereas, GI scores on the H side remained relatively stable, increasing slightly from baseline to day 21, but lagging significantly behind the NH side throughout the study (Figure 3). BOP scores on the NH side steadily rose from baseline to day 14, and then rapidly increased until day 21; whereas, the H side revealed no significant changes in BOP between baseline and day 7, a slight increase from day 7 to day 14, then a slight decrease to day 21 (Figure 4). SUL concentrations remained higher on the NH side throughout the study. SUL on the NH side, slowly increased from baseline until day 7, continued to rise until day 14, then rapidly increased until day 21; whereas, SUL on H side remained steady from the baseline to day 7, slightly increased between day 7 and 14, then continued to increase until day 21, but lagged significantly behind scores on NH side (Figure 5).

Figure 3. Mean GI Scores of Hygiene (H) and Non-Hygiene (NH) Sides Over Time

Figure 4. Mean BOP Scores of Hygiene (H) and Non-Hygiene (NH) Sides Over Time

Figure 5. Mean SUL Scores of Hygiene (H) and Non-Hygiene (NH) Sides over Time

Table I. Overall Mean Scores of GI, BOP and SUL on Hygiene Side (H) and Non-hygiene Side (NH)

Variable	Side	Baseline		Day 7		Day 14		Day 21	
		Mean	Std. Deviation	Mean	Std. Deviation	Mean	Std. Deviation	Mean	Std. Deviation
GI	H	0.15	0.21	0.17	0.21	0.23	0.26	0.29	0.47
	NH	0.10	0.16	0.53	0.31	0.71	0.27	1.04	0.48
BOP	H	0.04	0.06	0.05	0.08	0.10	0.14	0.08	0.10
	NH	0.06	0.10	0.11	0.14	0.19	0.19	0.62	1.34
SUL	H	0.01	0.02	0.01	0.02	0.03	0.03	0.06	0.06
	NH	0.01	0.02	0.02	0.02	0.05	0.05	0.15	0.08

N = 39

A comparison of the difference in mean scores of GI, BOP, and SUL are revealed in Table II. The Wilcoxon signed rank test statistics (d) for testing the significance of the difference in mean scores and the p-values are provided. Results suggest a significant difference in GI scores between sides ($p < .0001$, $p = .0001$, $p = .0001$) and BOP scores ($p = .011$, $p = .006$, $p = .0001$) at days 7, 14, and 21, suggesting that with the progression of disease, gingival inflammation, and bleeding increased significantly on the NH side. Data revealed a significant difference in SUL scores between sides at day 7 and 21 ($p=0.026$, $p=< .0001$); however, at day 14, there was no significant difference ($p= .053$).

Table II. Comparison of GI, BOP and SUL on Hygiene Side (H) with Non-hygiene Side (NH) Over Time.

Variable		Data Collection Intervals			
		Baseline	Day 7	Day 14	Day 21
GI NH-H	d	-8.64	30.38	32.79	30.69
	p	.084	**< .0001**	**< .0001**	**<.0001**
BOP NH-H	d	1.59	12.64	13.92	32.59
	p	.743	**.011**	**.006**	**<.0001**
SUL NH-H	d	-1.18	10.46	9.72	27.62
	p	.785	**.026**	.053	**<.0001**

N = 39   p = .05

d = Wilcoxon signed rank test statistic values, difference in mean scores

p = calculated as 2-tailed test

Bolded values indicate significance

data

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Data were analyzed to evaluate the correlation between SUL, GI, and BOP between sides. A Pearson correlation test revealed a moderate correlation on the NH side (r = .66, p<. 0001 and r = .61, p < .0001) and a low correlation on the H side (r = .49, p< .0001 and r = .45, p < .0001) (Table III). Results suggest that with progression of gingivitis, there is an increase in the strength of the positive correlation between SUL, BOP, and GI.

Table III. Correlation Between Sulfide Levels and Gingival Index, and Sulfide Levels and Bleeding on Probing on Hygiene (H) and Non-Hygiene Side (NH)

	Pearson Correlation Coefficient r			
	Gingival Index		Bleeding on Probing	
	NH	H	NH	H
Sulfide Levels	r = .66	r = .49	r = .61	r = .45
	p< .0001*	p< .0001*	p< .0001*	p< .0001*

N=39, * Indicates significance p= .05

Discussion

Analysis revealed a statistically significant positive correlation between SUL and GI and BOP on both H and NH sides; however, the strength of the association was higher for the NH side. These results suggest that H_2S in gingival crevicular fluid (GCF) increases with the severity of gingival inflammation. This finding supports the work of Solis-Graffar et al, who found a positive correlation between the GCF volume and H_2S production.[17]

In the present study, the increase of GI scores on the NH side at days 7, 14, and 21 represents a direct gingival response to the continued plaque accumulations on the teeth. In addition, the significant increase of SUL on the NH side may be due to the proliferation of gram-negative bacteria in plaque.[27] Since sulfide is a metabolic by-product of proteolytic gram-negative bacteria, increasing gram-negative bacteria would thereby increase sulphur by-products.[4] Whether sulfur by-product is a direct contributor to the disease process, or merely a correlate, needs further investigation.

GI scores on the H side did not change significantly until day 21. During this study, participants reported that they focused on their oral hygiene status and may have increased brushing and flossing time on the H side in order to compensate for plaque growth on NH side. Considering a split-mouth design was used, microbes may have been transferred from one side to another. In addition, participants cognizant of the ever-growing plaque accumulations on the NH side reported using their tongues to feel the difference between the two sides, which may have facilitated the transfer of bacteria. Although data seems to suggest a significant positive correlation between SUL, GI, and BOP scores on both sides, the strength of the correlation was stronger for the NH side.

Current findings complement the work of researchers who first suggested the use of BOP, instead of visible inflammation, as an objective indicator of early gingival pathology.[28] Data also supports the assumption that the GI is a reliable clinical parameter for early plaque-induced gingivitis, although transferring bacteria from one side to another may have affected GI scores. In contrast, present findings failed to support the work of researchers that suggested that GI is remarkably insensitive as a quantitative tool of early pathogenesis.[11]

Data revealed a statistically significant difference in SUL over time between the H and NH sides on days 7 and day 21; however, SUL concentrations at day 14 did not differ significantly. This data supports the work of Zhou, who found a statistically significant difference in SUL levels at days 5-6, 8, 12-14, 15-16, and 19, but not at days 10-12.[29] Both the work of Zhou and the present study demonstrated that SUL levels continued to rise throughout the experimental period and peaked at the end of the study despite the fact that at the end of week two, SUL concentrations were not significantly different between sides. Several theories may be advanced for this phenomenon. One explanation is that there is a decline

in anaerobic activity at approximately 10-14 days of the inflammation process whereby bacteria deplete their nutrients in the pocket. Another explanation may be related to host resistance. Perhaps around days 10-14, the strength of the host begins to exceed the capability of the bacteria.

Visual signs of inflammation are susceptible to subjective interpretation, can be masked by medications and smoking, and may not reflect the true periodontal status in areas inaccessible to visual inspection.[28] Therefore, monitoring SUL levels in addition to BOP and GI may more accurately reflect real-time disease activity within the sulcus. This interpretation needs to be validated by studying SUL levels in persons who use tobacco or take medications that may mask the clinical signs of inflammation. Identifying sites containing sulfides, in the absence of bleeding or visual gingival inflammation, may represent new and valuable diagnostic information.

It should be noted that data from this study suggest that SUL appear clinically at approximately the same time as BOP, but later than visible gingival inflammation. By day 21, SUL almost tripled from baseline, suggesting that SUL increase with the progression of gingival inflammation. Additional studies are needed to support claims of SUL as a valid indicator of early plaque-induced gingival disease and to determine how sulfide levels in the gingival sulci relate to ongoing gingivitis. The addition of microbial sampling with the evaluation of metabolic by-products of pathogenic bacteria may be one approach to diagnosing an active disease site.

Conclusion

Periodontal disease includes a group of inflammatory conditions of the periodontal tissue broadly categorized as gingivitis and periodontitis. Traditional clinical evaluations of the periodontium consist of an assessment of gingival inflammation, probing depth, loss of clinical attachment, bleeding on probing, and radiographic interpretation of the periodontium, which represent a retrospective analysis. Therefore, more reliable real-time clinical parameters are necessary to predict early disease activity prior to visual changes. The ideal assessment tool should be valid, reliable, time efficient, and easily used by clinicians.[30]

The purpose of this study was to evaluate the relationship between VSC and gingival health status, and to identify the capability of VSC in detecting early plaque-induced gingivitis in the absence of clinical signs. Given the findings, the following conclusions are offered:

The Diamond Probe/Perio 2000 System is able to detect sites with elevated sulfide levels.

Sulfide monitoring may be a useful real-time adjunctive indicator of early plaque-induced gingivitis.

Considering the overall design and outcome of this study, the following recommendations are made:

Increase the number and variety of participants to broaden the study population.

Include microbial sampling to monitor the presence of gram-negative anaerobic bacteria that may correlate with sulfides.

Correlate sites without bleeding and inflammation and positive sulfides.

Despite limitations of this study, the knowledge of sites with elevated sulfide levels will provide immediate feedback so that treatment options for targeting destructive bacteria may be considered. The continued investigation of the short and long-term relationship between sulfides and periodontal disease may enable the profession to develop a new clinical technique for assessing active tissue breakdown. Such a technique would allow practitioners to intervene before visible tissue destruction has occurred; therefore, the monitoring of sulcular VSC holds promise as a new periodontal assessment technique.

Acknowledgements

Notes

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