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Sulphur By-Product: The Relationship between Volatile Sulphur Compounds and Dental Plaque-Induced Gingivitis

Hui Zhou, BDS, RDH, MS; Gayle B. McCombs, RDH, MS;
Michele L. Darby, BSDH, MS; K. Marinak, DDS, MS

Abstract

The purpose of this study was to evaluate the relationship between volatile sulphur compounds (VSC) and gingival health status, and to monitor the changes in VSC in early dental plaque-induced gingivitis. Using an experimental gingivitis model, twelve subjects between 19 and 28 years old, with a healthy gingival status, refrained from brushing and flossing one randomly selected half of the mandibular arch for two weeks. At baseline and during six subsequent appointments, gingival inflammation (GI), bleeding on probing (BOP), and sulfide levels (SUL) were measured using the Gingival Index and the Diamond Probe/Perio 2000 System. The Spearman correlation was used to compare the relationships between SUL, GI, and BOP on the brushing (B) and non-brushing (NB) sides. Data on the NB side revealed a stronger correlation than on the B side. Wilcoxon rank sum was used to evaluate the differences between mean SUL, GI, and BOP scores on the B and NB sides over time. Results indicate that SUL were the first periodontal parameter to show a significant difference between sides. SUL were significantly higher on the NB side at 4 of the 6 data collection intervals; therefore, SUL may be associated with the initiation and progression of early plaque-induced gingivitis.

Keywords: Volatile sulfur compounds, plaque, gingivitis

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Introduction

Volatile sulphur compounds (VSC) are produced through the putrefaction activities of microorganisms on appropriate substrate components of dental plaque, debris adherent to mucous membranes, and saliva cellular elements, which include epithelial and blood cells.[1,2] VSC include hydrogen sulfide (H_2S), methyl mercaptan (CH_3SH), dimethyl sulfide (CH_3SCH_3), and dimethyl disulfide (CH_3SSCH_3).[3] H_2S and CH_3SH are primarily responsible for oral malodor, a condition perceived as a cosmetic problem; however, studies demonstrate, even at low concentrations, VSC are highly toxic to tissues[4,5] and may contribute to the etiology of both gingivitis and periodontitis.[6]

The evaluation of H_2S and CH_3SH production in the sulcus might be useful for monitoring the initiation and progression of gingivitis. Past studies have looked at the presence or absence of sulfides in the periodontal pocket and correlated those findings with the traditional clinical parameters of periodontal disease, which include gingivitis, probing depth, and bleeding on probing.[7,8] The present pilot study was built on past research to correlate gingival health and VSC concentrations with the progression of dental plaque-induced gingivitis.

2 mm or less

Researchers have long studied the localized toxicity of H_2S and CH_3SH in the oral cavity.[3-4,9-13] Ng and Tonzetich[4] indicated H_2S and CH_3SH cause a change in the structure of the crevicular epithelial barrier, which would permit an increase in accessibility of microbial substances to the underlying connective tissue layer, where they initiate a sequence of destructive inflammatory reactions. Horowitz and Folke[14] found H_2S in 89% of periodontal pockets with a depth of 4 mm or greater, and in only 6% of healthy crevices of 2 mm or less. Morhart et al.[15] identified H_2S in periodontal pockets that were 3 mm or deeper, most frequently located in the deepest portion of the pocket. The environment of a periodontal pocket favors both the proliferation of these anaerobes and their ability to produce H_2S, which damages epithelial cells and increases mucosal permeability. Yaegaki and Sanada[12] suggested VSC concentrations in mouth air from patients with probing depths of 4 mm or greater were higher than in subjects with a probing depth below 4 mm.

4 mm or greater

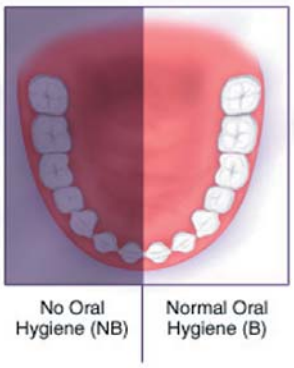
Persson et al.[16] reported the predominant VSC found in deep periodontal pockets was H_2S but significant amounts of CH_3SH also formed. Ratcliff and Johnson[6] suggested the ratio of CH_3SH and H_2S significantly increased in patients with periodontal disease in proportion to bleeding and probing depth. Yaegaki and Suetaka[2] focused on the analysis of thiol (-SH groups) and disulphide concentrations of VSC precursors in whole saliva and tongue coatings of periodontally involved mouths. They examined the correlation of VSC precursors with the indices of periodontal disease. Yaegaki and Suetaka's[2] data indicates the concentration of VSC precursors increased with the severity of periodontal disease, opening the door to future studies on the use of VSC as a diagnostic indicator of disease activity.

Methods and Materials

Prior to any treatment, each subject signed an informed consent, in duplicate, which had been approved by the Institutional Review Board of Old Dominion University. The protocol was discussed with each participant and medical histories were reviewed. Subjects with healthy gingiva who met the following inclusion criteria were enrolled:

1. Eighteen 18 years of age or older
2. Void of any mental and/or physical handicaps that would impair oral hygiene procedures
3. Free of orthodontic and prosthetic appliances
4. General good health
5. Non-smoker
6. Free of antibiotics within 30 days
7. Enrollment GI score of ≤ 1.0

Seven women and five men (N=12) between the ages of 19 and 28 were recruited from the Old Dominion University community. Since smoking is associated with increased incidence and severity of periodontal disease, all subjects were non-



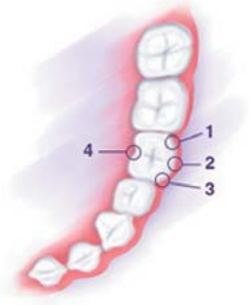
smokers to eliminate this variable. In addition, findings from Khaira et al.[17] suggest VSC levels were higher in smokers as compared to non-smokers. A split-mouth design, on the mandibular arch, was used to monitor the relationship between sulfide levels (SUL) and two periodontal parameters: the Gingival Index (GI)[18] and bleeding on probing (BOP), over a 14-day period. Each subject's mandibular arch was randomly divided: (NB) no oral hygiene (no brushing) on one quadrant and (B) normal oral hygiene measures (brushing) on the other quadrant. Data were collected on all teeth with the exception of third molars.

Baseline Data Collection Visit

A single trained and calibrated dental hygienist collected all data. Full mouth periodontal probing depths (PD) were obtained from 4 sites per tooth (distofacial, facial, mesiofacial, and mid-lingual) to establish baseline enrollment status. SUL, GI, and BOP scores were obtained from the same four sites. All subjects were given the same soft toothbrush and fluoride toothpaste TM without any added ingredients for control of gingivitis and tartar or whitening for their oral hygiene care on all areas of the mouth except the assigned NB quadrant. Subjects were told to withhold home care from assigned NB quadrant, but no attempt was made to modify subjects' daily oral self-care regime in the rest of the oral cavity.

Subsequent Data Collection Visits

Six subsequent data collection appointments were conducted over a 14-day period to assess SUL, GI, and BOP. Data were collected approximately every other day, except Saturday and Sunday; therefore, some data collection intervals/days were combined (Table 1). At the completion of the study, subjects received thorough oral and written home care instructions. Follow-up appointments were provided to ensure subjects return to baseline status.



Periodontal probing depth sites per tooth

1. Distofacial
2. Facial
3. Mesiofacial
4. Mid-lingual

Table 1. Data Collection Intervals (N=12).

Visit	Day
1	Baseline
2	Day 3-4
3	Day 5-6
4	Day 8
5	Day 10-12*
6	Day 12-14
7	Day 15-16, 19

*N=10

Equipment

The Diamond Probe/Perio 2000 System® is a dental device designed to detect sulfide concentrations of various forms (S, HS, H_2S, and CH_3SH) in gingival sulci (Figure 1). The system combines a conventional Michigan "O" style dental probe with a sulfide sensor, which measures PD, BOP, and SUL, simultaneously (Figure 2). The micro-sulfide sensor responds to sulfide ions and measures metabolic-products of mainly anaerobic bacteria and, indirectly, bacterial activity. The reaction of the sulfide ions with the sensor generates a measurable voltage that is proportional to the sulfide concentration. Since sulfides are continually cleared from the pockets by crevicular fluid flow, the presence of high sulfide levels indicates a high level of anaerobic bacterial activity.

Figure 1. Research Model of Diamond Probe/Perio 2000 System.

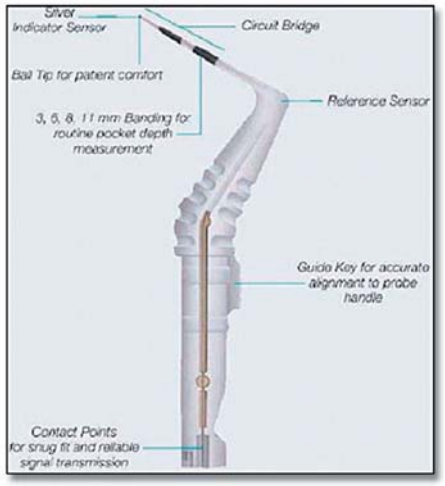


Figure 2. The Sulfide Probe Tip.

The Research Model of the Diamond Probe system was used according to manufacturer's instructions. The validity and reproducibility of obtaining the same reading within individual periodontal pockets on subsequent measurements, using different Diamond Probe sensors, was confirmed in data submitted to the FDA in the 510(k) submission. The probe tip was gently inserted into the base of the gingival sulcus and moved along with light pressure. As the probe moved along in the sulcus, the three periodontal parameters, SUL, GI, and BOP, were recorded. If the presence of sulfides were indicated above threshold (≥ .5), the light on the front of the display panel would change colors depending on sulfide concentrations and an audible tone would sound. The digital display would indicate the sulfide level from 0.0 (less than 10^{-7} mol of sulfide) to 5.0 (more than or equal to 10^{-2} mol of sulfide) in increments of 0.5.

BOP was recorded as either present (1) or absent (0). The GI was used to evaluate color, soft tissue consistency changes, and bleeding (score 0-3).

0 = Normal

1 = Mild inflammation - slight change in color, slight edema, no bleeding on probing

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

3 = Severe inflammation - marked redness or reddish-blue and enlarged, edema, ulceration, tendency to spontaneous bleeding

Statistical Analysis

Statistical analysis for this study included both descriptive and inferential tests. All measures were intervally scaled except for bleeding, which was nominally scaled. Prior to performing data analysis, scores on individual teeth were added together and mean scores were computed. Mean scores and standard deviations of SUL, GI, and BOP on B and NB sides are presented in Table 2. The Spearman correlation was used to determine the strength of association between SUL and the periodontal parameters. Wilcoxon rank sum was used to compare the difference of mean SUL, GI, and BOP scores between B and NB sides over time. All inferential analyses were made at the .05 level.

Results

Table 3 represents an analysis between mean SUL concentrations and GI scores; and mean SUL concentrations and BOP over time. Although slight, data revealed a higher degree of correla-

Table 2. Mean Score Comparison of Gingival Index (GI), Sulfide Levels (SUL), and Bleeding on Probing (BOP) on Brushing Side (B) & Non-Brushing Side (NB).

		Baseline (Visit 1)		Day 3-4 (Visit 2)		Day 5-6 (Visit 3)		Day 8 (Visit 4)		Day 10-12 (Visit 5)		Day 12-14 (Visit 6)		Day 15-16, 19 (Visit 7)	
		M	SD	M	SD	M	SD	M	SD	M	SD	M	SD	M	SD
GI	NB	.578	.251	.541	.262	.652	.283	.729	.177	.764	.260	.889	.165	.898	.234
	B	.616	.200	.646	.265	.637	.289	.664	.255	.554	.241	.634	.172	.771	.203
SUL	NB	.043	.058	.105	.095	.149	.071	.145	.078	.156	.123	.159	.116	.272	.127
	B	.033	.040	.073	.068	.074	.071	.067	.048	.086	.069	.071	.065	.100	.089
BOP	NB	.078	.094	.069	.089	.074	.111	.063	.079	.116	.090	.123	.104	.161	.099
	B	.074	.074	.071	.099	.030	.064	.024	.041	.024	.041	.027	.031	.060	.058

Table 3. Correlation between Sulfide Levels & Gingival Index and Sulfide Levels & Bleeding Over Time.

	Spearman's rho Correlation Coefficient r			
	Gingival Index		Bleeding on Probing	
	NB	B	NB	B
Sulfide levels	r = .39 p < .0001	r = .23 p = .04	r = .24 p = .03	r = -.12 p = .28

p = .05

Table 4. Comparison of Mean Sulfide Levels (SUL), Gingival Index (GI), and Bleeding (BOP) Scores between Sides.

		Visit 1 Baseline	Visit 2 Day 3-4	Visit 3 Day 5-6	Visit 4 Day 8	Visit 5 Day 10-12	Visit 6 Day 12-14	Visit 7 Day 15-16, 19
Variable	GI							
	NB-B	.707	.298	.885	.385	.087	**.007***	.147
	SUL							
	NB-B	.952	.486	**.014***	**.015***	.270	**.037***	**.002***
	BOP							
	NB-B	.859	.976	.318	.127	**.007***	**.005***	**.005***

p=.05
*bold indicates significance

tion between SUL and periodontal parameters on the NB side, which suggests that SUL increased along with bleeding and gingival inflammation.

Table 4 presents a comparison of mean SUL, GI, and BOP scores between sides over time. Data indicates a significant difference in SUL between sides at day 5-6 (p = .014), day 8 (p = .015), day 12-14 (p = .037), and day 15-19 (p = .002), which suggests SUL levels were significantly higher on NB side at these intervals. Data revealed a significant difference in mean BOP scores between sides at day 10-12, day 12-14, and day 15-19. There was a significant difference in mean GI scores only at one interval, day 12-14, which may suggest bleeding and the presence of SUL were more reliable indicators of gingival health than GI scores. The difference between sides appeared earlier and more frequently in SUL.

Figure 3, 4, and 5 demonstrate a visual representation of the difference between sides of SUL, GI, and BOP scores over time. SUL on the NB side were the first to show a significant difference (day 5-6) and remained higher throughout the study, increasing by 70% from day 12-14 until the end of study. BOP scores did not show a significant difference between sides until day 10-12, and GI scores were significantly different only at one interval (day 12-14).

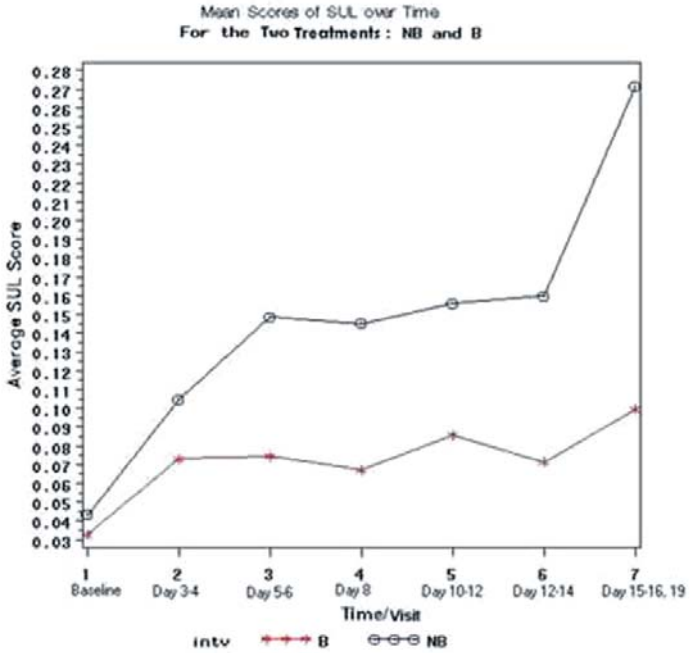


Figure 3.

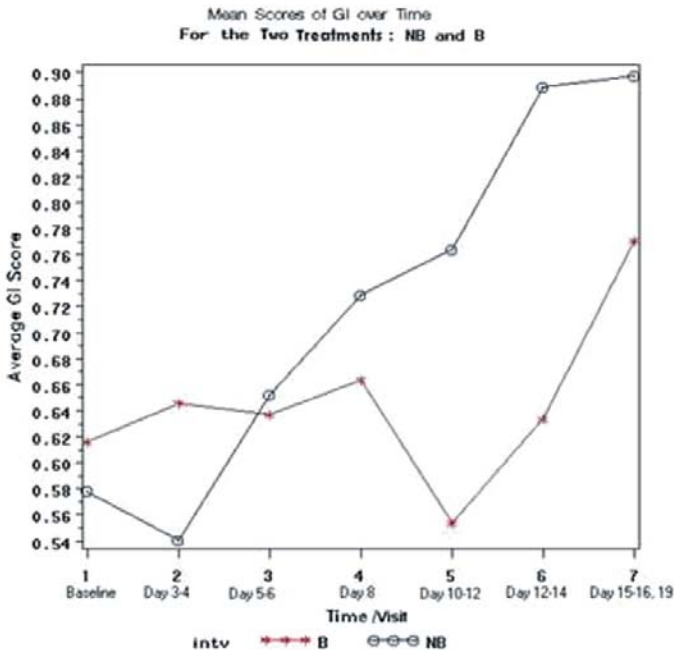


Figure 4.

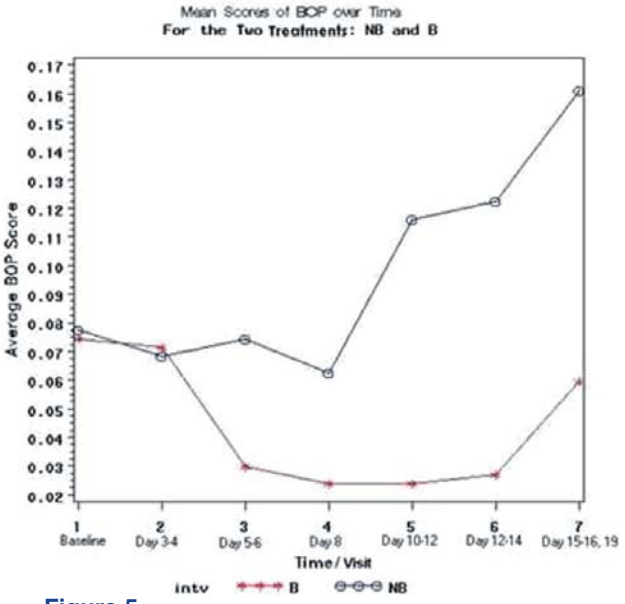


Figure 5.

Discussion

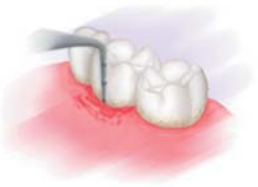
The relationship between SUL and periodontal disease is not yet fully understood, however, VSC have been shown to be toxic metabolites produced by microorganisms in the periodontal environment. During this study, SUL were the first periodontal parameter to show a significant difference between sides, and continued to increase throughout the study. Analysis of SUL and its relationship to GI and BOP revealed a higher degree of correlation on the NB side. This data suggests SUL increased with the severity of the gingival inflammation and bleeding, consequently, with the progression of dental plaque-induced gingivitis.

This finding supports the work of Solis-Gaffar et al.[13] who found a strong correlation between the gingival crevicular fluid (GCF) volume and H_2S production. The results of their study suggest that H_2S in GCF increase with the severity of gingival inflammation. The increase in SUL on the NB side may be due to the proliferation of Gram-negative bacteria in plaque. According to Persson et al.[19] sulfide concentrations are a metabolic by-product of proteolytic Gram-negative bacteria; thus, an increase in Gram-negative bacteria would result in an upsurge of sulfide by-products. However, more research is needed to determine how sulfide levels in the gingival sulci relate to ongoing gingivitis.

Analysis of SUL and BOP revealed a slight correlation between these two variables on the NB side, but no significant correlation on the B side. These correlations imply SUL may be involved in the progress of gingival inflammation. This observation supports the work of Yaegaki and Sanada[12] who found VSC concentrations were higher in mouth air from patients with BOP than patients with no BOP. The lack of correlation observed on the B side was expected and attributed to the minimal variation in BOP and SUL status measured in relative health.

The difference between SUL on both the NB and B sides revealed SUL appear more frequently and earlier in the disease process, nevertheless, not consistently. Increased SUL on the NB side implies SUL may be associated with the initiation and progression of early dental-plaque induced gingivitis, however, the reliability of this parameter needs further investigation.

Since traditional diagnoses of periodontal infections are retrospective in nature, the detection of SUL in the early, pre-bleeding stage of bacterial activity represents a new focus. Combining SUL monitoring along with the traditional clinical assessment may reduce the risk that periodontal infections will go undetected until evidence is apparent.



Based on these findings, the following assumptions are made:

1. SUL has a stronger correlation with GI and BOP on the NB.
2. SUL correlate with the severity and progression of gingival disease.
3. SUL may be involved in the pathogenesis of early plaque-induced gingivitis.
4. SUL analysis represents a real time observation of disease or health.
5. SUL monitoring should be considered as an adjunct to traditional clinical parameters for the assessment of disease activity.

Future research should replicate this study with an increased sample size of varying ages and levels of periodontal disease. Moreover, additional studies should be extended for a longer period of time and include microbiological sampling so SUL can be monitored under an extended bacterial challenge.

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About the Authors

Hui Zhou, BDS, RDH, MS

Dr. Hui Zhou is a dental hygiene practitioner in Williamsburg, VA. She received her BDS degree from Shanghai Railway Medical College of China in 1992 and her Master's Degree in Dental Hygiene from Old Dominion University in 2001.

e-mail: zhou_dental@yahoo.com.

Gayle B. McCombs, RDH, MS

Ms. Gayle McCombs is an Associate Professor and Director of the Dental Hygiene Research Center, School of Dental Hygiene at Old Dominion University in Norfolk, VA.

e-mail: gmccombs@odu.edu.

Michele L. Darby, BSDH, MS

Ms. Michele Darby is an Eminent Scholar, and Graduate Program Director, School of Dental Hygiene at Old Dominion University in Norfolk, VA.

e-mail: mdarby@odu.edu.

K. Marinak, DDS, MS

Dr. Marinak is a private practitioner and clinical instructor School of Dental Hygiene at Old Dominion University in Norfolk, VA.

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