The Reliability of A Colorimetric Test in Determining Gingival Inflammation

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ACCURATE ASSESSMENTS of prevalence, incidence, and severity are required to provide insight into preventive measures and treatment methods of periodontal disease. For this reason, indices have been devised to record disease changes. According to Ramfjord, an index should have precise, defined criteria for scoring that can be used under clinical conditions, be reproducible by other examiners in the same field, and be amenable to statistical analysis.[1] One of the indices used for recording severity of gingival inflammation is the measurement of the gingival fluid flow. Comprehensive literature reviews have been published regarding this subject.[2, 3]

The Gindex chemical analysis system is a recent product of Janar Company of Grand Rapids, Michigan. The company states that the Gindex test measures and, colorimetrically records the presence of hemoglobin from the gingival fluid.[4]

The chemical basis for the Gindex test is the Occult blood test, which in medicine has been used to detect fecal blood.[5] If hydrogen peroxide and orthotolidine dihydrochloride are added to saliva containing hemoglobin, the hemoglobin decomposes the hydrogen peroxide with the liberation of oxygen, oxidizing the orthotolidine to a blue colored derivative.[4]

The clinical basis for the Gindex test is the presence of gingival fluid from the sulci of inflamed gingiva, contributing hemoglobin to the saliva.

The Gindex saliva test is recorded by comparing the intensity of the blue color change with a color chart provided with the test kit. The color change is then quantitated using a somewhat arbitrary scale of from 0 to 100.

Although the Janar Company provides a literature review, for a test to be considered a useful index, it should be compared with other indices which have been proved to be good assessments of gingival disease. The purpose of the present study was to compare the Gindex test with the Gingival Index, by Löe and Silness,[6] and the intracrevicular gingival fluid collection technique as proposed by Löe and Holm-Pedersen[7] in the assessment of gingival inflammation.

MATERIALS AND METHODS

Patients seeking dental treatment at The University of Michigan School of Dentistry were utilized for this study. Additional subjects were obtained from the Institute for the Study of Mental Retardation and Related Disabilities Dental Clinic, Ann Arbor, and the Veterans Administration Hospital Services in Ann Arbor and Allen Park, Michigan.

Eighty-one subjects with ages ranging from 15 to 60 years of age, participated in this study. The subjects were selected to provide the following sample distribution according to the Gingival Index: 17—G.I. 0, 28—G.I. 1, 26—G.I. 2, and 10—G.I. 3. Besides the G.I., the Gindex saliva test and the crevicular fluid flow were recorded during the same patient visit.

Of the original 81 subjects tested, 11 were selected to compare test results obtained prior to hygienic phase procedures with those recorded 14 days later. Hygienic phase procedures included a prophylaxis, scaling, root planing, and oral hygiene instruction. These 11 subjects demonstrated varying degrees of gingival inflammation: 2—G.I. 1; 8—G.I. 2; and 1—G.I. 3.

Patients were required to have a minimum of 24 teeth and have no oral sores or abrasions. Written consent was obtained.

The Gindex saliva test was initiated before any clinical examination, by asking the subject to expectorate into a saliva cup provided with the kit. Later two drops of the subjects' saliva and two drops of the hydrogen peroxide "activating solution" were added to the vial containing the orthotolidine. The test results were recorded after the suggested 10 minute time period,[4] by comparing the color change with the color chart provided with the test kit. A numerical value from 0 to 100, based on the depth of color change, was recorded as the test score.

The gingival fluid was obtained from the mid-buccal and mesiobuccal surfaces of the six teeth used for the P.D.I.,[1] numbers 3, 9, 12, 19, 25 and 28. Each tooth was isolated with cotton rolls and air dried for 5 to 10 seconds. Strips of Munktell No. 3 filter paper were gently placed into the gingival crevice and left in place for 3 minutes, as proposed by Löe and Holm-Pedersen.[7]

Because of the difficulty in isolating the teeth used in the study, only two teeth were isolated and tested at one time. After 3 minutes, the strips were removed and permitted to air dry on microscope slides. The strips were then saturated with 2% ninhydrin and placed between microscope slides. When the filter paper strips were completely dried, the gingival fluid flow was measured using an ocular grid calibrated at 0.027889 mm per

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division. Square millimeter values were obtained by measuring squares or partial squares of darkly stained filter paper. The total mouth gingival fluid was expressed by obtaining the mean value of the mid-buccal and mesiobuccal areas of each test tooth, and then determining the mean value for the subject.

The Gingival Index scoring for each of the four units of the test teeth were recorded. The total mouth G.I. value for each subject was calculated by obtaining the mean value for each tooth, and the mean value for the subject.

The mean G.I. value, mean gingival fluid value, and the Gindex test score were used for statistical analysis to determine if any correlations existed among the three test measurements.

The following statistical tests were employed:

1. Student's "t" test comparing male and female populations.
2. Correlation coefficients between the G.I., crevicular fluid, and Gindex for the pooled populations.
3. Rank correlation coefficient between the G.I., crevicular fluid, and Gindex for the pooled populations.
4. Chi square analysis to demonstrate the association between the G.I. and the Gindex values.
5. Pair wise "t" test of changes in the G.I., crevicular fluid, and Gindex before and after prophylaxis treatment.
6. Pair wise rank statistics of changes in the G.I., crevicular fluid, and Gindex before and after prophylaxis treatment.
7. Correlation coefficient for the mean differences in G.I., crevicular fluid, and Gindex before and after prophylactic treatment.

Results

The sample was divided into males and females, to determine if there were any significant differences that could be attributed to sex. Table 1 indicates that a two-sample t test shows no significant differences between the mean values of males and females in any of the three tested parameters.

Therefore, the sample was pooled and tested for multiple correlations between the G.I., crevicular fluid, and Gindex scores. Table 2 indicates a statistically significant correlation ($P < 0.01$) between all parameters.

However, since the Gindex test results were not considered bivariate normal, nonparametric rank correlation coefficient tests were employed. Table 3 demonstrates a correlation between the three tested parameters ($P < 0.01$).

A chi square analysis (Table 4) shows that there was a significant difference between "low" and "high" Gindex scores (using 85 as the dividing score) for all of the G.I. categories.

The pre and post treatment group was tested for significant changes in G.I., crevicular fluid, and Gindex scores following hygienic phase procedures. A pair wise t – test for the pre and post treatment sample demonstrates a significant reduction in all three of the tested parameters (Table 5). A pair wise rank analysis also indicated that there was a significant reduction in the G.I., crevicular fluid, and Gindex values. Every subject demonstrated a reduction in G.I., and crevicular fluid values; and all but one subject showed a decrease in Gindex scores. For each variable, the sign test and the Wilcoxon rank – sum test on the differences between pre and post treatment values demonstrated that all of the differences were negative with both tests being significant, ($P < 0.001$).

Discussion

Attempts at measuring and quantifying gingival inflammation have been concerned with clinical assessment, crevicular fluid sampling, and microscopic evaluation. This investigation evaluated the effectiveness of

Table 2. *Correlation Coefficients*

	G.I.	FLUID	GINDEX
G.I.	1.000		
FLUID	0.5401	1.000	
GINDEX	0.5704	0.2963	1.000

r .01 = .2847

Table 3. *Rank Correlation Coefficients*

	G.I.	FLUID	GINDEX
G.I.	1.000		
FLUID	0.6570	1.000	
GINDEX	0.7869	0.4917	1.000

r .01 = .2880

Table 1. *Two – Sample T – Test Comparing Male and Female Populations*

	X MALE	X FEMALE	T.	SIGNIFICANCE
G.I.	1.4539	1.2885	0.93055	N.S. (0.3549)
FLUID	0.15208	0.17500	0.67727	N.S. (0.5002)
GINDEX	86.122	81.719	0.81688	N.S. (0.4165)

TABLE 4. *Chi Square Analysis Showing the Association Between the G.I. and Gindex Values*

		Gindex Test Values			
		below 85		above 85	
		#	%	#	%
G.I.	0	10	62.5	6	37.5
	1	11	37.9	18	62.1
	2	0	0	26	100
	3	0	0	10	100
total		21	25.18	60	74.82

Chi square = 25.921
D.F. = 3
p = 0.0000

TABLE 5. *Pair Wise T - Test for Pre and Post Prophylaxis Sample*

	Pre Tx. X	Post Tx. X	X Diff.	Std. Dev.	T - Stat.	Signif.
G.I.	1.7882	0.99645	0.79173	0.48769	5.3843	0.0003
FLUID	0.25573	0.05436	0.20136	0.34696	1.9249	0.0831
GINDEX	95.909	75.909	20.000	16.125	4.1138	0.0021

the Gindex saliva test by comparing test scores with the G.I. and the crevicular fluid sampling technique proposed by Löe and Holm-Pedersen.[7]

Brill[8–10] first noted that crevicular fluid sampling, by means of filter paper strips, might circumvent the subjectivity encountered when using the clinical parameters of color change and bleeding on probing. Microscopic evaluation, necessitating a biopsy, may be an extremely sensitive means of detecting inflammation, but is not suitable for routine clinical use.

This investigation demonstrated a low but statistically significant correlation between G.I. and crevicular fluid, G.I. and Gindex, and crevicular fluid and Gindex (see Tables 2 and 3).

Mann[11] conducted the first statistical study comparing crevicular fluid and clinical gingival inflammation. A correlation coefficient of 0.58 was found between the Parfitt index[12] and crevicular fluid flow values.

Egelberg[13] also studied the correlation between crevicular fluid flow and gingival inflammation. A rank correlation coefficient of +0.90 was observed between crevicular fluid flow and clinical inflammation. It should be noted that the author used an intracrevicular method where the strips were deeply inserted into the crevice until resistance was met. This sampling method could have provoked the low levels of flow noted in healthy gingiva and stimulated increased flow in the inflamed areas. Mechanical irritation from the sampling techniques will increase the vascular permeability, which is supposedly proportional to the degree of inflammation.[14]

Bjorn et al.[15] also utilized the "deep" intracrevicular sampling method and confirmed a statistically significant correlation between the degree of gingival inflammation (G.I.) and crevicular fluid flow.

Löe and Holm-Pedersen,[7] using a less irritating intracrevicular sampling method, found fluid flow to increase with the severity of gingival inflammation, but offered no correlation coefficients.

Other investigators[3, 16] also have noted significant correlations between clinical gingival inflammation and crevicular fluid flow.

When analyzing results for individual surfaces, Wilson and McHugh found a significant correlation between clinical inflammation and the crevicular fluid flow.[17] However, the authors showed a low correlation between the amount of mean fluid and the mean G.I. measured on the two selected teeth ($r = +0.18$). The poor correlation was explained by the variation in gingivitis at each of the surfaces being obscured by using mean values.

Orban and Stallard[18] compared crevicular fluid flow with clinical indices (P.D.I.[1] and O.H.I. −S.[19]), and biopsy evaluations. The mean crevicular fluid flow measurements did not correlate with the inflammatory status of the whole mouth, nor did the fluid measurements correlate well with the individual biopsy areas.

Gray et al.[20] performed a clinical and histologic study of the relationship between crevicular fluid and gingival inflammation. Although the G.I. and histologic grading of inflammation correlated well (+0.16), the quantity or the presence or absence of crevicular fluid was unreliable as a predictor of the level of inflammation.[21]

Daneshmand and Wade[21] also demonstrated a weak

correlation between the histologic index and crevicular fluid (+0.34), and a weak correlation between the G.I. and crevicular fluid (+0.148).

Many investigators have alluded to the fact that crevicular fluid sampling techniques may produce varying results.[7, 14, 15, 22]

Any physical irritation to the crevicular lining can create a state of altered vascular permeability, and although it may be considered proportional to the inflammation, it may not depict an accurate assessment of the gingival health.

Although in the sampling technique used in this investigation, care was taken to avoid deep penetration into the gingival crevice, the possibility exists that varying the sampling method introduced a source of error.

The fact that investigators have reported different correlation coefficients suggests that although they are supposedly measuring gingival inflammation due to bacterial plaque, actually they may be recording altered vascular permeability due to mechanical irritation by the testing device.

It has been reported that hormonal alterations may affect gingival vascular permeability.[23-25] Female sex hormones may increase vascular permeability, especially when gingival inflammation is already present. Most studies[23-25] dealing with this subject employed the Brill intracrevicular sampling technique, which could have stimulated fluid flow through mechanical irritation.

It was beyond the scope of this investigation to obtain a menstrual cycle history from the female subjects, but as Table 1 indicates, there was no apparent difference between males and females in any of the three parameters tested.

A possible source of error in using the ninhydrin staining method of crevicular fluid collection is salivary contamination. Ninhydrin, being a stain specific for amino acids, will detect salivary as well as crevicular fluid amino acids. In this investigation, only the deeply stained portions of the filter paper strips were recorded as crevicular fluid flow.

Although the Gindex saliva test records hemoglobin from crevicular fluid, there are potential inherent problems with the test kit. The eye droppers supplied with each kit are designed to deliver a "standard" 0.05 cc drop; however, ropey saliva is not easily delivered in "standard" drops. In these cases, more hemoglobin may have been placed in solution with the orthotolidine and the hydrogen peroxide, producing a false high score.

Most of the positive Gindex scores were above the 75 to 85 range, on the 0 to 100 scale. The Janar Company states that the linear relationship of the blue color change and hemoglobin concentration terminated at score 85; and that scores above this value may not be an accurate representation of gingival bleeding.

This factor would produce a basic positive correlation between the gingival inflammatory parameters, but may be partially responsible for the rather weak nature of the correlation between the Gindex scores and crevicular fluid flow ($r = +0.49$). This however would not explain the relative high correlation between the Gindex test and the G.I. ($r = +0.79$).

It is possible that the concentration of the orthotolidine is too great to demonstrate a more even distribution of Gindex scores. This may explain why so many "positive" scores were above the 75 to 85 range.

Perhaps the primary advantage of the Gindex saliva test kit lies in patient motivation. Because the test is a colorimetric reaction performed with the patient's saliva at chairside, the patient may be able to visualize his or her own improvement in gingival health. The design of this study did not include an evaluation of the patient's motivation; consequently this factor remains untested.

One of the potential uses for the Gindex saliva test is to depict changes in gingival health following oral hygiene instruction or periodontal treatment. To test whether the Gindex system would demonstrate significant positive results, as a sample, 11 subjects were tested, given a prophylaxis, and tested again 14 days later. The results of this section indicated that there was a significant reduction in the G.I., crevicular fluid flow, and Gindex scores following treatment.

Gwinnett et al.[26] using the Harco flow meter, demonstrated a reduction in crevicular fluid flow following a dental prophylaxis. Although a greater reduction was noted following a second prophylaxis, this indicates that crevicular fluid flow may be used to monitor the tissue response to prophylaxis treatment.

Suppipat et al.[27] also noted a decrease in crevicular fluid flow at 14 days following scaling and polishing. The reduction in fluid flow must reflect the tissue's return to health at the level of strip placement. The time period after treatment at which the sample is taken, is important, since varying amounts of tissue injury to the crevicular lining and the subjacent connective tissue fibers during the procedure may cause delayed healing with subsequent crevicular fluid production. Collection of gingival fluid too soon after a prophylaxis may reflect inflammation from loss or partial loss of the crevicular lining, and not to inflammation associated with dental plaque.

The Gindex test demonstrated a reduction in all but one of the subject's examined. Because the test is sensitive and frequently demonstrates high values, the actual amount of reduction may not produce enough color change to influence the patient. This problem possibly could be compensated for by reducing the concentration of the orthotolidine.

The clinical applicability for the Gindex saliva kit must be discussed with reference to the test's extreme sensitivity. The Janar Company advertises the test to be a useful diagnostic aid for detecting early gingivitis in the general dental practice.[4] Findings from this investigation suggest that the Gindex system will detect very early gingivitis, and thus support the use of the kit for

this purpose. Because the test records relatively high numerical values for both minor and severe inflammation, one would question the test's ability to discriminate the severity of inflammation. However, in comparison with the G.I., the Gindex showed a rank correlation coefficient of +0.7869; which suggests that the Gindex test does discriminate well compared with the G.I. This could be interpreted as meaning the Gindex is an accurate assessment of disease, and could be used as an index. On the other hand, it could mean that the G.I. does not delineate the middle ranges of inflammation.

A problem encountered when evaluating or comparing inflammatory indices is that there is no universal accurate standard index with which to compare. Biopsy inflammatory cell counts are accurate but not practical, crevicular fluid techniques are difficult to standardize, and clinical indices are subjective.

Daneshmand and Wade summarized their experience with comparing indices by suggesting that subjective clinical assessment using any of the advocated indices is often easier and less time consuming than gingival fluid collection and inflammatory cell counts, and would be just as accurate.[21]

The Gindex saliva test is easily performed and is not time consuming, making its use attractive. However, more testing should be attempted using different concentrations of the orthotolidine to determine if the Gindex test could better discriminate levels of inflammation. In spite of these shortcomings, the Gindex saliva test may be a useful diagnostic aid in dental practice for detecting gingival inflammation.

Summary

This investigation was undertaken to compare the Gindex saliva test scores with the Gingival Index scores and crevicular fluid flow scores, as proposed by Löe and Holm-Pedersen.

Eighty-one patients were tested using the Gindex chemical analysis kit. Crevicular fluid was collected from the six teeth advocated by Ramfjord, followed by a clinical assessment of these teeth using the G.I.

Of the original 81 subjects tested, 11 were given a prophylaxis consisting of scaling, root planing, polishing, and oral hygiene instruction; and were retested 14 days later.

Results of the two-sample t test show that there were no significant differences between males and females in any of the three tested parameters. The sample was pooled and tested for multiple correlations between the G.I., crevicular fluid, and Gindex scores. Nonparametric rank correlation coefficient tests indicated a statistically significant correlation between the three parameters.

A pair wise "t" test for the pre- and post-treatment sample demonstrated a significant reduction in the three parameters. A pair wise rank analysis also showed a significant reduction in the G.I., crevicular fluid, and Gindex scores.

Conclusions

Within the limits of this investigation, it may be concluded that:

1. There was a statistically significant correlation among scored values of the Gindex saliva test, the Gingival Index, and crevicular fluid flow.
2. The Gindex test scores demonstrated a significant reduction following a prophylaxis, consisting of scaling, root planing, polishing, and oral hygiene instruction.
3. The Gindex chemical analysis may be a useful diagnostic aid for detecting the presence or absence of gingival inflammation in dental practice.

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Announcements

AMERICAN BOARD OF ORAL MEDICINE

The American Board of Oral Medicine will examine candidates for certification in conjunction with the Annual meeting of the American Academy of Oral Medicine at the Hotel del Coronado in San Diego, California on April 26, 1979. Further details may be obtained from the Secretary, Dr. William K. Bottomley, 12711 Glen Mill Road, Potomac, Maryland 20854.

UNIVERSITY OF CONNECTICUT SCHOOL OF DENTAL MEDICINE

The University of Connecticut School of Dental Medicine announces the following continuing education courses:

TITLE: Rational Drug Selection
DATE: December 6, 1978
FACULTY: DR. HAROLD GAYNOR, ALEX A. CARDONI, M.S., DENNIS J. CHAPRON, M.S.

TITLE: Orthodontics as Adjunctive Therapy in Periodontal Practice
DATES: December 7–8, 1978
FACULTY: DR. RAVINDRA NANDA, DR. DAVID ROMEO, DR. MANUEL TORRES-DIAZ, DR. JEFFREY BERT

TITLE: Physiology and Pathology of Oral Tissues
DATES: January 5, January 19, February 2, February 16, 1979
FACULTY: DR. LESLIE CUTLER, DR. JAMES YAEGER, DR. JOHN NALBANDIAN

TITLE: Periodontal Prosthesis with Special Emphasis on Orthodontics and Adjunctive Tooth Movement
DATES: January 18–20, 1979
FACULTY: DR. MORTON AMSTERDAM, DR. ROBERT VANARSDALL

TITLE: Four-Handed Dentistry in Endodontics and Periodontics
DATES: January 24, 1979
FACULTY: DR. PHILIP LEVIN, DR. EARNEST SPIRA

TITLE: Clinical Periodontology & Periodontal Prosthesis
DATES: March 22–23, 1979
FACULTY: DR. JAN LINDHE, DR. STURE NYMAN

TITLE: Periodontics for the Dental Hygienist
DATES: March 28, 1979
FACULTY: DR. HAROLD HORTON, DR. DAVID GELB

TITLE: Management of Medical Emergencies
DATES: April 25–26, 1979
FACULTY: MR. GREGORY METCALF AND STAFF

TITLE: New Knowledge of Nutrition for Dentists
DATES: June 21–22, 1979
FACULTY: DR. FELIX BRONNER

TITLE: Periodontics In General Practice
DATES: June 27–28, 1979
FACULTY: DR. PAUL ROBERTSON

For further information contact: Dr. Harold M. Gaynor, Associate Dean for Continuing Education, University of Connecticut School of Dental Medicine, University of Connecticut Health Center, Farmington, Conn. 06032

HOSPITALIZED CARE WORKSHOP ANNOUNCED

The American Academy of Periodontology, through its Hospital Care Committee, takes pleasure in announcing a 1-day workshop-conference on periodontics in the hospital dental program, Friday, December 1, 1978 at the V.A. Hospital, 1st Avenue and East 24th Street, New York, NY.

This is the first time that all periodontists who are hospital-associated will have an opportunity to meet, discuss, and demonstrate their hospital programs in periodontics and oral medicine.

The program will include detailed information relating to intern-resident training in periodontics, in-service courses for visiting dental staff, special periodontal training sessions for hygienists, nurses and volunteers associated with the periodontal section of the hospital dental department.

Printed material will be provided to all participants to serve as guidelines for their programs. Outstanding speakers and clinicians will serve as moderators and discussion will be encouraged to make the workshop productively effective. When you write, please list all the members of your staff who plan to attend. If you wish to be included in luncheon plans, there is a $5 fee.

Attendance must be limited and participants will be registered according to the order of receipt of applications. Applications and checks should be sent to: Dr. S. J. Ewen, 107-21 Queens Blvd., Forest Hills, New York 11375.

Address communications to: Marilyn C. Holmquist, Executive Secretary and Editor, or Jean Pierson, Associate Editor, 211 E. Chicago Ave., Room 924, Chicago, Illinois 60611