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Comparison of various plaque parameters in individuals with poor oral hygiene

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The control of plaque formation on tooth surfaces would appear to be a major goal of preventive dentistry. Several subjective scoring procedures for estimating plaque have been described. In the present investigation, stained and unstained plaque scores were compared with various physical characteristics of plaque such as dry weight, wet weight, microscopic count, absorbance of dispersed plaque suspensions, nitrogen and carbohydrate content. An institutionalized population that exhibited moderate to severe gingivitis and heavy plaque accumulations was studied. Significant correlations between the gravimetric measurements and microscopic count with either gingivitis scores or the unstained plaque scores were found. The stained plaque score showed no correlation with plaque weight or gingivitis scores in this population.

In animals, different types of dental plaque predispose to either caries (Keyes 1968) or periodontal disease (Socransky 1970). The control of plaque formation and/or accumulation would therefore appear to be a major objective of preventive dentistry. A variety of potential therapeutic agents, i.e. antiseptics (Löe and Rindom Schiött 1970), antibiotics (Mitchell *et al* 1967, Volpe *et al* 1969) and enzymes (Caldwell *et al* 1971, Lobene 1971, Keyes *et al* 1971) have in recent years been tested clinically for their ability to reduce or eliminate plaque accumulation. The efficacy of these therapeutic agents in reducing plaque has been estimated by various clinical scoring systems (Ship, Cohen and Laster 1967). In the present investigation one such scoring system (Kobayashi and Ash 1964) was compared with various physical characteristics of plaque such as dry weight, wet weight, microscopic count, nitrogen and carbohydrate content. The correlation coefficients of plaque score and of these physical and chemical plaque parameters with gingivitis scores were determined so as to see which characteristic of plaque showed the best statistical relationship to gingivitis. These measurements were performed in

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Table I

Frequency Distribution of Gingival Scores, Plaque Scores and Dry Weight Amongst Institutionalized Individuals

Score	Frequency Distribution – Initial Time Gingivitis*	Plaque*	Dry Weight	Weight
0				0 mg
1.0			1	2
1.5	2		4	4
2.0	1		5	6
2.5	10		1	8
3.0	7	2	2	10
3.5		6	1	12
4.0		5	3	14
4.5		3	0	16
5.0		4	2**	40
				50
			1**	60

* Ramfjord, J. Periodont. 1959, Kobayashi & Ash, Angle Ortho, 1964

** large calculus deposits

conjunction with a clinical trial of topical kanamycin as a means of controlling plaque accumulation (Loesche *et al* 1971).

Material and Methods

Subjects: Twenty institutionalized residents of the Plymouth State Home and Training School, Northville, Michigan served as subjects. Eight females and 12 males ranging in age from 13 to 32 years were included in the study. These neurologically impaired and mentally retarded individuals had at the initial visit moderate to severe gingivitis, high plaque scores and in some instances massive calculus deposits (Table 1).

Plaque measurements: The six index teeth, i.e. 3, 9, 12, 19, 25, and 28 recommended by Ramfjord (1959) were scored for plaque (Kobayashi and Ash 1964) and gingivitis (Ramfjord 1959). The buccal or labial plaque was stained with bismark brown prior to scoring in 10 individuals, whereas in the other 10 subjects, it was estimated with the naked eye without any disclosing agent. One examiner with experience in this method of plaque scoring made all the estimates of the stained plaque and some of the estimates of the unstained plaque. A second examiner, newly trained, made the remaining scores of the unstained plaque. The same criteria for area coverage were used by both examiners.

Buccal or labial surface deposits, including calculus if present, were then removed from the index teeth and collected in small aluminum boats. For six subjects, the boats were weighed at chairside within one minute after collection and a wet weight obtained. For all individuals, at all occasions, plaque was returned to the parent laboratory and separated into two portions of known weight. One portion was dried at 95° C until constant weight was obtained. This weight was used to determine the dry weight of the entire plaque sample. The second portion was suspended in 10 ml of distilled water and was homogenized by exposing it to 30 seconds of sonic vibration, i.e. maximum intensity from the standard tip of a sonifier (Heat Systems). Aliquots of this suspension were used for carbohydrate analysis by the anthrone procedure (Osler 1965) and nitrogen measurements by a Nessler procedure (Osler 1965). In addition, the microscopic count was determined in a Petroff Hauser counting chamber (Aranki *et al* 1969). In later trials 0.001 M ethylene diamine tetraacetate (EDTA) was added to the water, because it reduced clumping of bacteria as observed under the microscope. The absorbance of this plaque suspension was read at 420 mμ in a Spectronic 20 spectrophotmeter. As absorbance is proportional to turbidity, this procedure was a simple way of measuring bacteria and other insoluble material present in plaque.

Each subject was seen 4 times over a 12 week period, i. e. at 0, 4, 8, and 12 weeks. During this period oral hygiene measures were suspended. After plaque and gingivitis were scored at the initial visit, and the plaque removed from the reference teeth, all teeth were given a thorough oral prophylaxis. At

Table II

Correlation of Plaque Scores with Gingivitis Score and Other Plaque Parameters

	Plaque Stained		Plaque Unstained			
Examiner	1		1		2	
Comparison of Plaque Score With	n*	r**	n*	r**	n*	r**
Gingivitis Score	32	0.04	14	0.64 #	24	0.50 #
Dry Weight	33	0.16	14	0.59 #	23	0.78 #
Nitrogen	27	0.21	8	0.94 #	18	0.35
Carbohydrate	32	0.12	14	0.25	–	–
Wet Weight	–	–	–	–	23	0.75 #
Microscopic Count	–	–	–	–	21	0.55 #

n* = Number of observations r** = Correlation Coefficient
Correlations which are sinificant $p<0.05$

weeks 4, 8, and 12, 4 week old plaque was scored, removed and quantitated on the 6 reference teeth. At week 4, after the plaque was scored and removed from the reference teeth, either 5 % kanamycin in orabase or orabase alone was applied topically to the dental gingival surfaces of all teeth for a 5-day period. The results of antibiotic treatment have been reported elsewhere (Loesche *et al* 1971).

The various statistical analyses were done by an IBM 360/67 computer using CONSTAT, a console-oriented statistical computing program, developed for use on the Michigan Terminal System.

Results

Correlation of Plaque Scores with Gingivitis Score and Other Plaque Parameters.
Stained plaque scores are the usual means of estimating plaque (Ship, Cohen and Laster 1967). The values obtained indicate the area of the teeth that have stainable plaque adhering to their surfaces. Plaque can also be estimated with the naked eye (Löe 1967). Table 2 shows the correlations obtained when stained and unstained plaque scores were compared with the various parameters using data obtained from all 4 sampling periods, i.e. at 0, 4, 8 and 12 weeks. In addition, Table 2 shows the correlations obtained when the scoring was performed by two different examiners. The stained plaque scores obtained by the experienced examiner did not correlate with gingivitis scores, dry weight, nitrogen content or

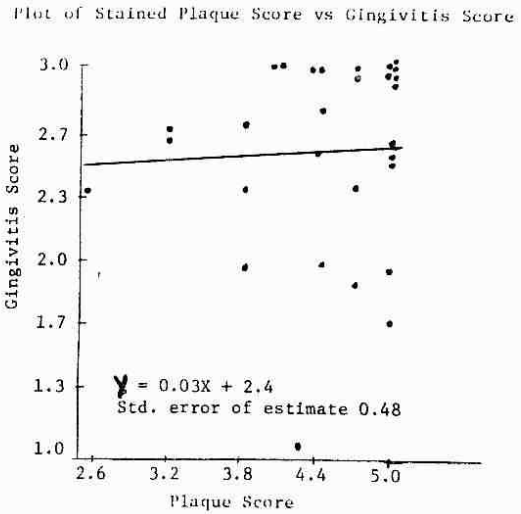

Fig. 1. Scatter plot showing relationship between stained plaque score and gingivitis score.

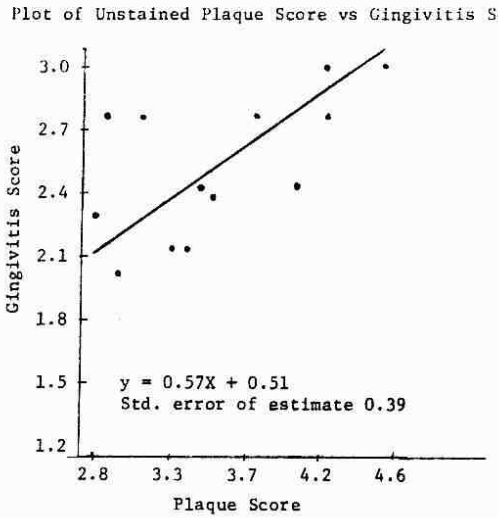

Fig. 2. Scatter plot showing relationship between unstained plaque score and gingivitis score.

carbohydrate content of the plaque. However, the same examiner, applying the same subjective criteria to be unstained plaque, was able to obtain significant correlations, i.e $p<0.05$, between plaque scores and gingivitis scores, dry weight and nitrogen. The inexperienced examiner was also able to obtain significant correlations between the unstained plaque scores and gingivitis scores and dry weight. At this time two new measurements were introduced, i.e. wet weight and microscopic count in the EDTA solution. Both of these correlated significantly with the unstained plaque scores, i.e. $p<.01$. Scatter plots of plaque scores vs. gingivitis scores for examiner 1 are shown in Figures 1 and 2. When stained plaque scores were used, there was a stacking of points at the boundary of the scoring system, i.e. 11 of the 32 observations fell on the line $x = 5.0$ (Fig. 1). Thus in this population, the upper limits of the stained plaque scores were easily reached with a third of the values clustered at the maximum. This skewness prevented any correlation between stained plaque scores and gingivitis scores. The unstained plaque score, which is not as dependent on surface area for its values, overcame this difficulty of stacking of points at the boundary. Unstained plaque scores were distributed between 2.8 and 4.6 with no value falling at $x = 5.0$ (Fig. 2). This distribution permitted significant correlations between unstained plaque scores and gingivitis scores regardless if the examiner was experienced or newly trained.

Correlation of Gingivitis Scores with Various Plaque Parameters.

A gingivitis score measures the degree of inflammation by arbitrarily chosen subjective criteria. It can yield information that is valuable in both an epidemiological and clinical situation. When the correlation matrices were determined, gingivitis scores correlated significantly with dry and wet weight, microscopic count and the unstained plaque score (Table 3). Plaque nitrogen and carbohydrate content as well as the stained plaque scores were not significantly associated with the gingivitis scores.

Correlations of Various Plaque Parameters with Dry Weight.

The preceding results demonstrated that in

Table III

Correlation of Various Plaque Parameters With Gingivitis Score

Comparison of Gingivitis Score With	n*	r**	95% confidence Limits	Significance
Plaque Score				
stained	23	0.12	−0.27 to 0.5	$p>.10$
unstained	28	0.65	0.35 to 0.81	$p<.001$
Dry Weight	50	0.44	0.2 to 0.65	$p<.01$
Wet Weight	21	0.57	0.2 to 0.8	$p<.01$
Microscopic Count	20	0.57	0.2 to 0.8	$p<.01$
Nitrogen	36	0.19	−0.15 to 0.47	$p>.10$
Carbohydrate	33	0.03	−0.3 to 0.38	$p>.10$

n* = Number of observations. r** = Correlation Coefficient

Table IV

Correlation of Various Plaque Parameters With Dry Weight

Comparison of Dry Weight With	n*	r**	95 % Cofidence Limits	Significance
Plaque Score				
stained	24	0.27	−0.13 to 0.6	p>.10
unstained	27	0.84	0.67 to 0.92	p<.001
Gingivitis Score	50	0.44	0.2 to 0.65	p<.01
Wet Weight	22	0.92	0.8 to 0.95	p<.001
Microscopic count	25	0.84	0.65 to 0.92	p<.001
Absorbance	52	0.72	0.57 to 0.82	p<.001
Nitrogen	57	0.51	0.28 to 0.66	p<.001
Carbohydrate	44	0.47	0.2 to 0.67	p<.01

n* = Number of observations. r** = Correlation Coefficient.

this population gravimetric measurements of plaque correlated significantly with the unstained plaque score and gingivitis score. Additional statistical analyses were performed comparing the various measurements obtained on 4 week old plaque collected at weeks 4, 8, and 12 with dry weight. The initial visit or 0 time values were not included, because the age of the plaque was not known and, in some instances, considerable amounts of calculus were present. Calculus was not evident in the 4 week old plaque samples. The data presented in Table 4 shows that wet weight,microscopic count, absorbance, nitrogen and carbohydrate correlated significantly with dry weight. Of these measurements, wet weight was the easiest to perform and with a correlation coefficient of r = 0.92 would be a reliable substitute for dry weight. A scatter plot of wet vs. dry weight is shown in Figure 3. The wet weight must be determined immediately, as the plaque sample rapidly dehydrates, i.e. 20 to 40 % weight loss within 1 hour. The microscopic count determined in the 0.001 M EDTA suspension showed a high correlation with dry weight. This measurement, however, has a large uncontrolled variable in the amount of clumping of organisms that can be observed under the microscope. The EDTA and sonification just prior to counting significantly reduced the amount of clumping and the size of the clumps. For this reason, only the microscopic counts obtained in the presence of EDTA were used in the statistical analysis. Despite the significant correlations of microscopic counts with plaque weight, plaque scores and gingivitis scores (Tables 2, 3, 4), this measurement is time consuming and too error prone to be a routine procedure. In an attempt to find a routine procedure, the turbidity or absorbance of the sonified EDTA plaque suspensions was determined. Absorbance readings are simple and quick and with an r value of 0.72 might be a useful way of estimating plaque mass. Large numbers of samples can be processed at the convenience of the investigator. However, calculus will not disrupt readily, thereby restricting the value of this measurement to samples in which calculus is not present. The plaque dry weight would be comprised of carbohydrate, nitrogen and inorganic materials.

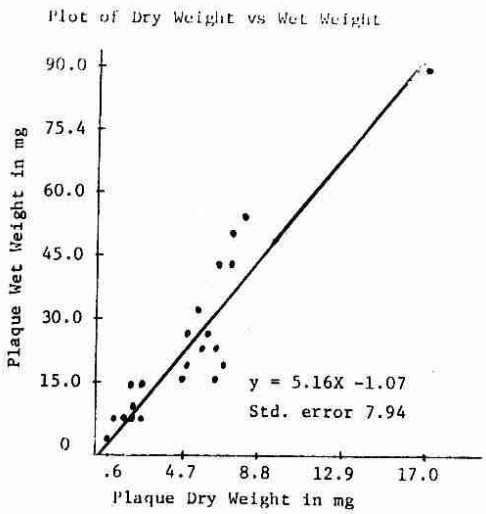

Fig. 3. Scatter plot showing relationship between plaque dry and wet weigh.

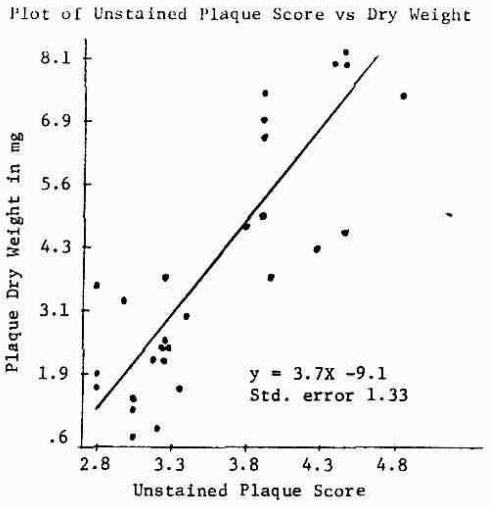

Fig. 4. Scatter plot showing relationship between unstained plaque score and dry weigh.

Thus the positive correlation between dry weight and plaque nitrogen and/or carbohydrate (Table 4) was to be expected.

In these 4 week old plaques there was no significant correlation of stained plaque scores with dry weight. However, when the same scoring criterion was applied to the unstained plaque, a highly significant correlation was found, i.e. $r = 0.84$. A scatter plot showing this relationship is presented in Figure 4.

Correlation of Various Plaque Parameters with Wet Weight.

Wet weight, if determined immediately, was approximately 4 times the dry weight (Fig. 3). It showed a better correlation than dry weight with the gingivitis score (Tables 4, 5). For this reason, wet weight may be the preferred measurement to use for the estimation of plaque mass.

Discussion

In the present experimental situation some of the components of plaque were measured and the various interrelationships between plaque scores, gingivitis scores, wet and dry weight, and composition of plaque determined. Significant correlations between the gravimetric measurements and either gingivitis scores or the unstained plaque score existed. Wet weight would appear to be the preferred measurement for estimating plaque mass because it was easier to obtain than a dry weight, correlated closely with dry weight and the unstained plaque score, and showed a higher correlation with gingivitis scores than did dry weight.

The unstained plaque score has the advantage of simplicity and of not disturbing plaque *in vivo*. The high correlation of this score with dry weight, wet weight and gingivitis was probably due to the ability of the naked eye to discern only plaque that was thick enough to be visualized. Therefore it provided an approximation of plaque mass rather than surface area. The limitations of the unstained plaque score would be that it was subjective and nonlinear. The unstained plaque score because it imposes a fixed boundary at its upper limit would not be reliable when there is massive amounts of plaque, such as in the initial visit reported in this study (Table 1). This fact can be noted by observing that r values in Tables

Table V

Correlation of Various Plaque Parameters With Wet Weight

Comparison of Wet Weight With	n*	r**	95 % Confidence Limits	Significance
Plaque Score unstained	17	0.82	0.57 to 0.92	$p<.001$
Gingivitis Score	21	0.57	0.2 to 0.80	$p<.01$
Dry Weight	22	0.92	0.8 to 0.95	$p<.001$
Microscopic Count	19	0.72	0.42 to 0.87	$p<.001$

n* = Number of observations. r** = Correlation Coefficient

3, 4, and 5 unstained plaque were higher than the corresponding r values in Table 2, where the findings of the first visit were included. Ideally, in the assessment of plaque mass, both a weight measurement and an unstained plaque score should be obtained. However, in instances where very little plaque is present, it may be impractical to remove and weight the plaque. In this circumstance an unstained plaque score might be the only measurement possible. Löe and Rindom Schiött (1970) have used unstained plaque scores in following the effectiveness of a chlorhexidine mouthrinse as a plaque controlling agent. The plaque index (Löe 1967) is predicated on measuring plaque thickness rather than area coverage and from their report would appear to be a reliable method of assessing the effects of a therapeutic agent on plaque mass and associated gingivitis.

The stained plaque scoring systems of the type employed in this investigation were designed for evaluating oral hygiene and efficacy of various cleansing agents and devices such as toothbrushes. A disclosing solution stains all tooth surface films and can provide accurate measurements of the tooth surface area which is covered by plaque. When surface area of plaque is the parameter under investigation these scoring systems would be perhaps the measurement of choice.

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