Clin Oral Invest (2006) 10: 140–144
DOI 10.1007/s00784-006-0036-5

ORIGINAL ARTICLE

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A new in vivo method for measuring caries activity with a colorimeter

Received: 26 October 2005 / Accepted: 10 January 2006 / Published online: 24 February 2006

Abstract The aim of this in vivo study was to assess the association between caries prevalence and changes in mineralization measured with a colorimeter (Color Compare CC 400, JENOPTIK, Jena, Germany). After a clinical examination (defs/DMFS, initial caries lesions), an area of a deciduous tooth was etched in each of the 35 children (Ø=8.11±2.41 years) with 37% phosphoric acid gel for 1 min. Immediately after, this demineralization was stained with 2% aqueous methylene blue and the red/green/blue spectrum measured with a colorimeter. Twenty-four hours later, the remineralization of this area was measured following the same staining procedure. Color measurements were clearly reduced after 24 h, indicating remineralization, and they correlated highly with the age of the children (Spearman correlation coefficient r=−0.48, p=0.004). Correlations between the number of initial caries lesions in the deciduous and permanent dentition and color measurements after demineralization were statistically significant (r=0.41 and 0.37, p=0.02 and 0.045, respectively). The difference between the first and second measurements correlated significantly with the number of initial caries lesions in the permanent dentition (r=0.42, p=0.02). The values after artificial demineralization correlated with the number of initial lesions for the permanent (r=0.368, p=0.045) and deciduous (r=0.408, p=0.015) dentition. This resistance to artificial demineralization had stronger correlation coefficients with the caries incidence than the caries experience and initial lesions, which are considered to be the most valid caries predictors. In conclusion, these data suggest that the degree of demineralization after etching and its changes with time could be associated with caries parameters. Its use in prospective clinical trials on caries activity could be a successful approach.

Keywords Caries activity · De- and remineralization · Colorimeter

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Introduction

Despite evidence from the last decades that the prevalence of dental caries has drastically decreased, the disease is far from eradicated [4, 5, 11, 15]. In the countries which experienced this decline, dentistry is now dealing with a new generation of patients in which the majority has a rather slow, progressive state of the disease. Therefore, further caries prevention should be risk-adjusted in this population. In spite of extensive research, the sensitivity and specificity of individualized caries risk diagnosis are still suboptimal. One of the current scientific problems in cariology is that currently there is no single trustworthy test to predict caries development [6, 7, 13]. Models, which include several factors can increase the accuracy slightly, but no reliable mode for calculating these different factors can be generated. In a summary of the literature on caries risk, Hausen [6] points out that the present methods have not reached the desired predictive power. Thus, a high number of wrongly classified children result in high costs of treatment for unnecessarily intensive prevention or restorations.

Owing to the difficulties in finding a proper risk model and the difficult or unfeasible calculation of the individual risk factors, it is proposed that instead of calculating caries risk, caries activity should be used for caries prediction, as caries activity represents the sum of all risk factors and their interdependencies. Caries activity is deduced from the behavior of the de- or remineralization of the dental tissue, as the measurement of this reflects the caries activity of an individual at the time of the assessment. Measuring de- and remineralization directly on the tooth makes it possible to consider the sum of all the individual factors that act on the

tooth. It can be confirmed whether multiple factors influencing caries, such as sugar intake, bacteria, saliva, and fluoride, operating as a whole, result in demineralization and therefore in caries, or whether mineral ions are able to precipitate from solution so that there is no caries activity. Thus, a single test would allow individual factors that produce caries to be measured as a whole. In the present study, both the de- and remineralization potential in vivo was studied for the assessment of caries activity.

Based on a method for initial caries diagnosis developed by Axamit [3], Möller et al. [9] introduced a method to assess de- and remineralization in vivo. The proposed method consists of minimal etching and tooth dyeing. The uptake of dye, which is related to mineral loss and/or mineral gain, was measured with a color scale. It was used in further studies on remineralization speed [9, 12, 14]. The main problem with this method was the subjective color evaluation using a color scale. This could be improved by the use of a colorimeter (Color Compare CC 400, JENOPTIK, Jena, Germany), which measures the color digitally.

Materials and methods

The color measurement device "Color Compare CC 400" was developed for differentiation, recognition, and control of red-green-blue values of a colored surface. The object is irradiated with light at a wavelength between 380 and 780 nm. The reflected spectrum is divided by photo diodes into 512 frequency ranges and different red-green-blue (RGB) color values are measured by an integrated microchip with reference to standardized light (D 65, JENOP TIK). The color measurement is then analyzed by the equipment and the results are displayed as RGB values between 0 (minimum) and 4,500 (maximum). Before every measurement, a calibration of the device against a custom-made ceramic guide is necessary. The measurements are taken by pressing the tip of the device on the test area. An acoustic control signal tells the operator that the measurement has been taken. The colorimeter was easily linked to a computer for data transfer into an Excel database.

Examination of the reproducibility and repeatability of color and white measurement (pilot study)

To control the quality of the colorimeter, the standard blue color fields S3040 R90B, S3050 R90B, S3060 R90B, and S3065 R90B (color card "Mathys Mix," Mathys S. A., Zelem, Belgium, certified according to NCS Quality Level 2, 1997) were used to perform several measurements. For the repeatability, four precalibrated sequences of 50 consecutive measurements with an interval of 5 min were carried out by one examiner. Afterwards, a second sequence of 50 measurements was conducted by four different examiners to test the reproducibility.

All measurements were recorded in the same room under identical physical conditions using artificial light (2×58 W fluorescent light tube, Osram, Germany).

Determination of the remineralization rate in vivo (main study)

The study was approved by the Greifswald University Ethics Committee on Research and conducted in accordance with the ethical principles described by the Helsinki Declaration.

Subjects

A sample size calculation (nQuery Advisor 4.0 software) showed that using a two-sided Fischer's z test for the null hypothesis, the Pearson correlation coefficient would have 80% power to detect a p value of 0.450 when the sample size was 30 to 40 subjects.

A total of 35 patients of the Pediatric Department of the Greifswald Dental School (20 males and 15 females, 3 to 11 years old, $\varnothing$=8.11±2.41 years) were selected according to different clinically diagnosed levels of caries activity. Their parents were informed about the purpose of the study and they gave written consent for their children to participate. The inclusion criteria included subjects that had at least one clinically healthy vestibular surface of a deciduous tooth. Exclusion criteria were lack of cooperation and serious oral or general diseases.

Clinical examination and measurements

One experienced dentist carried out all clinical examinations where defs/DMFS scores [16] and initial carious lesions were recorded in 35 children (3–11 years old). Subsequently, a clinically intact vestibular surface of a deciduous tooth was selected upon which to etch a site (area=6 mm^2) for 1 min with 37% phosphoric acid gel (Email Preparator GS, Vivadent, Germany). Immediately after, the etched area was stained with 2% aqueous methylene blue for 3 min, then carefully dried with a cotton pellet to absorb excess dye-solution, and the RGB values were measured ten times in succession with a calibrated colorimeter (Color Compare CC 400, JENOPTIK). After 24 h, the same area was measured again using the staining procedure mentioned above.

Statistical analysis

The results of the study were statistically analyzed using the computer program SPSS (Version 12.0). For testing reproducibility, the 95% confidence intervals and the Bland–Altman procedure were used from every individual

sequence of 50 in vitro measurements. From the ten de- and remineralization in vivo measurements, mean values were calculated for the different colors (red, green, and blue) and used for further analyses. The RGB value was calculated as the sum of these three colors. The remineralization rate was defined as the difference between the RGB values after artificial demineralization and after 24 h. Positive values indicate a decrease in RGB values while negative values indicate an increase from the first to second appointment. The repeatability and reproducibility were assessed with the *t* test after calculating mean values and standard deviation. The correlations between parameters were checked with the Spearman–Rho test ($p \leq 0.05$). Gender did not correlate with any of the parameters (age, defs, DMFS, initial lesions, and RGB values), which allowed for a common analysis of male and female.

Results

Repeatability and reproducibility of the colorimeter

The total range of the color measurement device Color Compare CC 400 was 0–4,500 units for red, green, and blue values. The variations of measurements on the standardized color card Mathys Mix were negligible. In 62 measurement with rows of 50 measurements each, the 95% confidence interval (Fig. 1) was ±15 U only or less, and therefore differed by less than 1.5%. This excellent reproducibility was also confirmed by the Bland–Altman procedure.

The comparison with measurements of the standardized white Spectralon specimen showed a slightly higher variation but 80% of the measurements varied less than 2% from the first value. The maximum confidence interval of variation was ±2.8%. The interexaminer variation was as low as the intraexaminer variation.

Bivariate analysis of caries prevalence and color changes after demineralization

Color measurements were clearly reduced after 24 h and they correlated highly with the age of the children (Spearman correlation coefficient $r=-0.48$, $p=0.004$; Fig. 2). Correlations between the number of initial caries lesions in deciduous and permanent dentitions and color measurements after demineralization were statistically significant ($r=0.41$ and 0.37, $p=0.015$ and 0.045, respectively; Table 1). The difference between the first and second measurement correlated significantly with the number of initial caries lesions in the permanent dentition ($r=0.42$, $p=0.02$; Table 1) and tended to correlate with the number of initial caries lesions in the deciduous dentition, but this correlation was not significant ($r=0.30$, $p=0.083$; Table 1).

The red values increased significantly with age ($r=0.385$, $p=0.022$) while the remineralization rate (difference of the total values before and after artificial demineralization) was reduced ($r=-0.496$, $p=0.002$).

The defs correlated statistically and significantly with the number of initial caries lesions in the deciduous dentition ($p=0.019$, $r=0.394$; Table 1), with the changes in the blue values ($p=0.007$, $r=0.449$), and the RBG values of the first colorimeter measurement after artificial demineralization ($p=0.015$, $p=0.408$). The correlation between the defs and the remineralization rate, i.e., the difference between the first and second measurement (RGB_1–RGB_2) was slightly weaker ($p=0.083$, $r=-0.297$). The number of initial carious lesions in the permanent dentition correlated strongly with the demineralization ($p=0.045$, $r=0.368$) and with the remineralization ($p=0.022$, r =−0.416). Although the color values showed a strong correlation among each other, the strongest correlation was found between their sum (RGB values) and the caries parameters (Table 1).

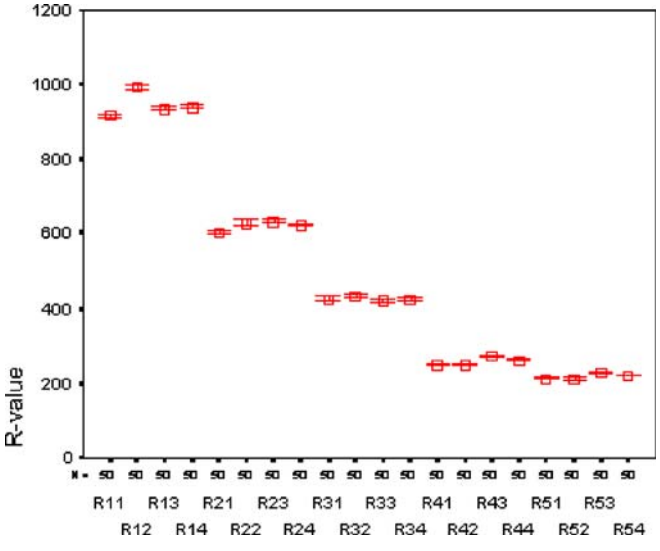

Fig. 1 Variation (95% confidence interval) of four series in repeated measurements each (1–4) for five different shades of red (R1–R5)

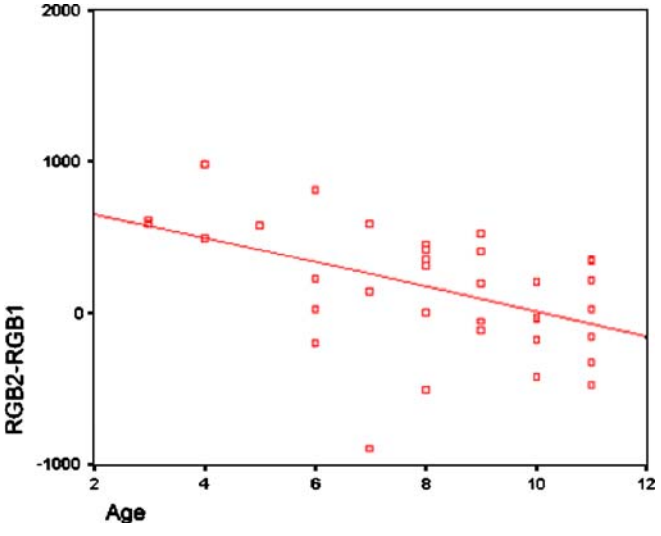

Fig. 2 RGB changes after 24 h remineralization depending on age

Table 1 Correlations (*r* values: Spearman–Rho correlations coefficient and according *p* value) between age, defs and DMFS values, number of initial caries lesions in deciduous (ci) and permanent teeth (Ci), and the resistance to artificial demineralization for the red (R), green (G), blue (B), and their sum (RGB) in the first and second measurement

		Age	defs	ci	DMFS	Ci
ci	*r*	0.029	0.394			
	p	0.870	0.019			
DMFS	*r*	0.493	0.179	0.181		
	p	0.006	0.344	0.338		
Ci	*r*	0.120	0.327	0.470	0.160	
	p	0.570	0.078	0.009	0.398	
R_1	*r*	0.385	0.158	0.332	0.174	0.227
	p	0.022	0.365	0.051	0.356	0.228
G_1	*r*	0.272	0.147	0.274	−0.020	0.283
	p	0.114	0.400	0.051	0.917	0.129
B_1	*r*	0.111	0.042	0.449	0.208	0.347
	p	0.524	0.809	0.007	0.271	0.060
RGB_1	*r*	0.270	0.106	0.408	0.164	0.368
	p	0.117	0.545	0.015	0.385	0.045
RGB_2	*r*	−0.306	−0.19	0.061	0.034	−0.168
	p	0.074	0.274	0.726	0.858	0.374
$\Delta RGB_2–RGB_1$	*r*	−0.477	−0.267	−0.297	−0.203	−0.416
	p	0.004	0.122	0.083	0.282	0.022

Discussion

The results of the pilot study show excellent reproducibility and repeatability for the colorimeter used in this study, which justifies the use of this system for the proposed method, especially as no differences between various operators were found. The correlation between the caries prevalence and the color measurements after etching and after 24 h also supports the hypothesis that differences in color uptake are associated with the demineralization–remineralization balance resulting in different caries levels.

A 1-min demineralization period seems to be effective for the method as previous investigations [9, 12, 14] using a similar acid-etching-staining procedure also achieved positive results following this procedure. The 1-min demineralization was performed here in all participants without any problems. At the second appointment, no remaining dye-solution was clinically detectable in any patient.

A 24-h measurement interval was chosen because previous studies [9, 12, 14] recorded measurable differences after this time period. Recent studies [1, 2, 8] showed that the degree of remineralization in erosive lesions is already considerable and measurable even after one hour.

The present study suggested that the resistance to etching and the degree of change in staining over 24 h differed from one individual to the other. Similarly, it seems reasonable to suggest that the resistance to acids from dental plaque and the remineralization potential after an acid attack also differ among children. The balance of de- and remineralization, which represents the caries activity, might be measurable with this new method.

Up to now, past caries experience has represented the most relevant single parameter of future caries prediction [13]. This includes active initial carious lesions [10], which are in fact already representatives of the disease they are supposed to predict. Compared to caries experience and the diagnosis of active initial lesions, the advantage of the proposed method is the early identification of caries activity before cavitated lesions or even initial lesions are clinically detectable. This is truer to the intentions of primary prevention than waiting for initial lesions or even cavitated defects to occur. In addition, a method like this would be less susceptible to interexaminer differences than common caries risk parameters such as caries lesions, plaque, or gingivitis. In summary, the procedure presented using colorimeter analysis of an etched test site and its subsequent remineralization behavior suggests that the degree of demineralization achieved by etching and the subsequent changes with time could be an instrument to measure caries activity. The results of the current study offer a possible starting point for future longitudinal investigations.

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