

## Can tooth color be used as part of caries risk assessment? A cross-sectional study

Tooth color and dental caries burden

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### Abstract

**Aim:** This study aimed to investigate whether the colors of the maxillary central incisor (MC1) and the maxillary first premolar (MP1) can serve as indicators to estimate dental caries risk.

**Material and Methods:** A total of 140 healthy dentistry students aged 18-25 years participated in this cross-sectional study. The MC1 and MP1 were polished with a polishing brush for 20 seconds. After polishing, in room light, L\*, a\*, b\*, chroma, and hue values were measured from three different regions of the teeth with the VITA Easyshade spectrophotometer device. After color evaluation, dental caries burden was determined according to the Decayed, Missing, and Filled Teeth (DMFT) index. A questionnaire addressing demographic features, dietary habits, and smoking habits was also administered. Spearman's correlation tests and multiple linear regression analyses were conducted.

**Result:** No significant correlation was found between DMFT scores and L\* values. The DMFT index was positively correlated with the a\*, b\*, and chroma of the MC1 but was negatively correlated with hue. In terms of the MP1, no significant correlation was detected between DMFT and color parameters. Smoking and tooth-brushing had significant effects on MC1 b\* and chroma and on MC1 a\*, b\*, and chroma, respectively. No significant effect of confounding factors was observed on MP1 L\*, a\*, b\*, chroma, or hue.

**Discussion:** This study concludes that dental caries risk may be predicted with color values of the MC1. However, the same does not apply for the MP1. The ability to predict dental caries by evaluating the color of the MC1 shows the potential for color to be used as a part of caries risk assessment.

### Keywords

Color, Correlation of Data, Dental Caries, Operative Dentistry

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## Introduction

Dental caries are affected by many environmental factors and constitute one of the most common chronic diseases in the world [1]. The quality and quantity of saliva, amount of consumed carbohydrates, fluoride intake, and hygiene habits of the patients are some of the relevant environmental factors [2]. In addition, differences in the enamel and dentin structure may affect one's susceptibility to dental caries [3, 4].

One of the most important factors affecting the aesthetic appearance of teeth is color. Color can be defined by dimensions such as hue, light value, and chroma. Value is the amount of light reflected from an object. Chroma, on the other hand, expresses the saturation and intensity of the hue. In other words, there is an inversely proportional relationship between chroma and light values [5]. The absorption of light reduces the amount of light reflected on the human eye, resulting in a low light value and high chroma. While the enamel is capable of reflecting light in high amounts due to its highly inorganic structure, the dentine absorbs light more due to its highly organic structure and therefore it has high chroma [6]. Changes in the tooth surface that affect both color and dental caries susceptibility may be associated with the accumulation of bacterial biofilm [7].

The fact that the composition and thickness of the tooth structure can affect both dental caries and color may indicate the existence of a relationship between these two factors. However, to our knowledge, no study in the literature has examined this relationship to date, and it is therefore necessary to conduct research in this regard. The present study aims to determine the association between the color of the maxillary central incisor (MC1), the color of the maxillary first premolar (MP1), and caries burden as measured by the Decayed, Missing, and Filled Teeth (DMFT) index. The null hypotheses of the study were as follows:

- (1) Color of the MC1 is not a predictor of caries burden.
- (2) Color of the MP1 is not a predictor of caries burden.

## Material and Methods

### Setting and Design

This health center-based cross-sectional study was conducted among dentistry students at Sütçü İmam University in Kahramanmaraş, Turkey, in June and July 2020. The sample size was calculated with the statistical program G\*power 3.0.10 (<http://www.gpower.hhu.de/>). With 80% power, 0.05 alpha error, and 0.24 effect size (effect size predetermined based on a study group of 20 individuals), the necessity for 133 samples was determined. Inclusion criteria for baseline screening included being a healthy dentistry student between the ages of 18 and 25 years and having at least one non-restored MC1 and one non-restored MP1. Individuals meeting any of the following exclusion criteria were not considered for initial screening: (1) individuals treated with bleaching, (2) individuals with any health problems that could be an obstacle to dental hygiene, (3) individuals with internal staining (for reasons such as fluorosis, tetracycline, etc.) in both of their MC1 or MP1 teeth, and (4) individuals with a hereditary disease that may affect the structure of the teeth.

### Blinding Procedures

To prevent measurement bias, the researchers were blinded

to each other (each researcher individually examined each participant). Color and dental caries evaluations were carried out by Ö.H. and K.T.T. separately. The data obtained by these researchers were recorded in different Excel files (Microsoft Excel, Office 2013, USA) and sent to another researcher (E.M).

### Color and Dental Caries Examinations

Before the examinations, all examiners were initially trained and calibrated according to the WHO Basic Surveys Calibration Protocol for caries detection, coding findings, and recording, consisting of a theoretical training session followed by oral examination of five subjects (not part of the study sample) at Sütçü İmam University. Adequate standardization with a kappa value of 0.82 for caries and kappa value of 0.80 for color was obtained; 10% of examinations were duplicated and the percentage of agreement was 79% and 83% for caries and color, respectively.

Three different dimensions of color were measured objectively: L\*, a\*, and b\*. L\* indicates the light value, taking a numerical value between 0 (black) and 100 (white). As chromatic coordinates, a\* and b\* indicate the green-red axis and blue-yellow axis, respectively. In this respect, the formula  $C = [(a^*)^2 + (b^*)^2]^{0.5}$  is used in calculating chroma [5].

Color evaluation was carried out by Ö.H. The right MC1 and right MP1 (the left ones were used in the absence of right teeth or when there was a restoration in the right tooth) were polished with a polishing brush (Stoddart, Germany) for 20 seconds. After polishing, the teeth were dried with cotton pellets. In room light, the L\*, a\*, b\*, chroma, and hue values were measured from three different regions of the teeth using the VITA Easyshade spectrophotometer device (VITA Zahnfabrik, Bad Säckingen, Germany).

An intraoral dental examination was performed for each subject by the same researcher (K.T.T.) using a sterile dental shepherd's hook explorer and mirror. Caries evaluation was performed by visual exploration and digital panoramic radiography. In cases in which caries detection was challenging, the bitewing radiography technique was used to support the panoramic radiography. After the tooth was dried, each tooth was recorded as decayed (D), missing (M), or filled (F) and caries burden was then determined according to the DMFT index. Teeth filled or missing for a non-carious reason were not recorded and were checked according to the individual's history. Additionally, third molar teeth were not recorded.

### Questionnaire

A structured questionnaire was formulated by Ö.H. The language of the questionnaire was Turkish and it was translated into English. The questionnaire had adequate reliability with a Cronbach alpha coefficient of 0.716. Demographic features [age, gender (male OR female), family income (none OR 0-300 US\$ OR 300-700 US\$ OR 700-1400 US\$ OR 1400 US\$ and more), hygiene habits [such as tooth-brushing (once weekly OR once daily OR twice daily and more) and tongue-brushing (yes OR no), flossing (none OR once weekly OR once daily OR twice daily and more), and use of a mouthwash (none OR once weekly OR once daily OR twice daily and more)], and dietary habits [drinks (juice OR buttermilk OR cola OR coffee OR tea OR water) and foods (protein OR carbohydrates OR vegetables OR fruit) usually consumed] as well as amounts of sugar used in

tea (none OR 1 teaspoon OR 2 teaspoons OR 3 teaspoons and more per serving) and smoking habits (yes OR no) were asked and recorded.

**Statistical Analysis**

The jamovi program (version 1.0.4; accessed at <https://www.jamovi.org>) was used for statistical analysis. The normality of data distribution was checked using the Shapiro-Wilk test. The correlation between dental caries and color parameters was calculated by Spearman test. Multivariate linear regression models were applied for all parameters, adjusting for any confounders. The omnibus ANOVA test was conducted to calculate the equality/inequality of the regression coefficients in multiple regression. Significance was set at  $p \leq 0.05$ .

**Results**

A total of 145 dentistry students were included in the initial

screening and five were excluded from the analysis because of previously restored MC1 and MP1 teeth. In total, 140 dentistry students, including 50 men and 90 women with a mean age of  $19.6 \pm 1.47$  years, met the eligibility criteria and were included in this study.

Descriptive analyses of caries and color parameters for all survey questions are shown in Table 1. No significant effect was observed on DMFT or MC1 L\* in the linear regression analysis ( $p > 0.05$ ). It was observed that smoking had a significant effect on MC1 b\* and chroma ( $p < 0.05$ ), with values being higher among students who smoked. Tooth-brushing had a significant effect on MC1 a\*, b\*, and chroma, with these values being lower among those who brushed their teeth more frequently. Beverage habits had a significant effect on MC1 hue, with this value being highest among those who consumed coffee. However, no significant effect was observed on MP1 L\*, a\*,

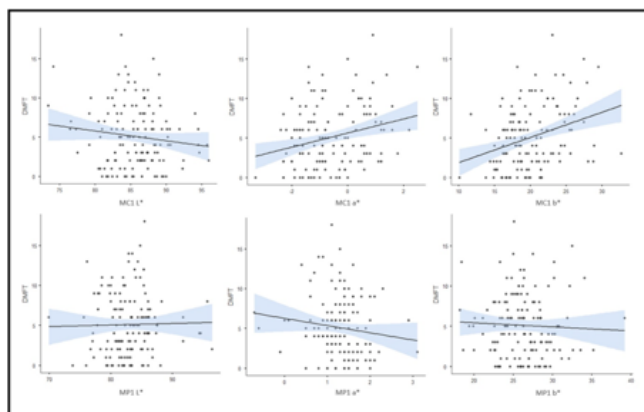
**Table 1.** Descriptive analysis of caries and color parameters (n=140)

Parameters	DMFT	MC1					MP1					
		L*	a*	b*	Chroma	Hue	L*	a*	b*	Chroma	Hue	
Gender	Male (n=50)	4.48±3.56	85.5±3.68	-0.31±1.11	20.7±4.02	20.8±4.00	91.4±3.52	83.0±2.95	1.24±0.61	26.1±3.35	26.1±3.36	87.4±1.30
	Female (n=90)	5.46±4.01	85.6±4.17	-0.75±1.13	19.7±3.76	19.8±3.72	92.7±3.79	82.9±4.38	1.32±0.49	26.5±3.41	26.5±3.42	87.2±0.98
Family income	None (n=2)	7.00±2.85	86.1±2.12	-0.05±1.20	20.5±4.67	20.6±4.60	90.5±3.46	84.1±4.81	0.50±1.56	22.4±4.24	22.5±4.24	89.2±3.89
	0-300 US\$ (n=23)	4.78±3.54	84.3±3.07	-0.32±1.20	20.6±3.85	20.7±3.84	91.4±3.38	81.9±3.73	1.47±0.74	27.0±4.19	27.1±4.21	87.0±1.45
	300-700 US\$ (n=49)	5.22±4.47	85.4±3.78	-0.50±1.11	20.5±3.52	20.6±3.47	91.8±4.03	82.4±3.41	1.38±0.41	27.1±3.07	27.1±3.07	87.1±0.79
	700-1400 US\$ (n=40)	5.55±3.66	85.7±4.66	-0.63±1.22	20.2±4.31	20.3±4.29	92.4±3.62	83.2±3.56	1.14±0.41	25.3±2.56	25.3±2.57	87.4±0.92
	1400 US\$ and more (n=5)	1.20±1.79	84.7±2.67	-1.34±0.68	17.2±2.74	17.3±2.68	94.8±2.72	79.2±2.88	1.50±0.19	26.2±2.28	26.2±2.31	86.7±0.42
Smoking habit	Yes (n=22)	4.82±3.79	85.3±3.85	-0.78±0.86	19.3±3.31	19.4±3.26	92.7±2.83	83.4±3.58	1.23±0.49	26.5±2.89	26.6±2.88	87.4±1.15
	No (n=118)	5.16±3.90	85.6±4.02	-0.56±1.18	20.2±3.97	20.3±3.94	92.2±3.89	82.9±3.98	1.31±0.55	26.3±3.48	26.3±3.49	87.2±1.10
Tooth-brushing habit	Once weekly (n=6)	4.67±3.08	83.8±4.22	0.88±0.93	26.8±4.44	26.9±4.44	88.4±2.17	81.3±3.44	1.73±0.84	27.9±3.6	27.9±3.63	86.5±1.21
	Once daily (n=46)	3.93±3.33	85.9±4.64	-0.47±1.13	20.1±3.58	20.2±3.57	91.9±3.45	83.2±4.68	1.36±0.63	26.5±4.14	26.5±4.15	87.2±1.27
	Twice daily and more (n=88)	5.75±4.08	85.6±3.51	-0.76±1.09	19.6±3.59	19.7±3.54	92.7±3.83	83.0±3.43	1.23±0.44	26.1±2.93	26.2±2.94	87.3±0.99
Dental floss habit	None (n=85)	4.94±3.70	85.8±3.68	-0.53±1.23	20.3±4.26	20.4±4.22	92.1±4.10	82.7±3.93	1.32±0.53	26.2±3.43	26.3±3.44	87.2±1.01
	Once weekly (n=39)	5.50±5.28	86.2±4.08	-0.67±0.87	20.3±3.17	20.3±3.13	92.2±2.76	83.3±4.42	1.38±0.40	28.6±3.45	28.7±3.46	87.3±0.71
	Once daily (n=14)	5.64±3.63	84.1±4.67	-0.52±0.92	19.8±2.92	19.9±2.91	91.8±2.77	82.0±3.41	1.35±0.69	25.4±2.96	25.4±2.97	87.0±1.69
	Twice daily and more (n=2)	2.50±3.54	83.8±0.56	-1.05±0.91	18.0±1.20	18.0±1.13	93.4±3.11	83.5±0.35	1.30±0.14	28.8±2.62	28.8±2.62	87.4±0.42
Mouthwash habit	None (n=95)	4.80±3.79	85.6±3.82	-0.53±1.13	20.1±3.94	20.2±3.90	92.2±3.84	82.6±3.97	1.31±0.57	26.2±3.58	26.2±3.59	87.2±1.15
	Once weekly (n=28)	5.93±4.07	85.8±4.47	-0.72±1.27	19.7±4.02	19.8±3.98	92.8±3.80	84.2±3.96	1.30±0.34	27.0±2.90	27.0±2.91	87.3±0.60
	Once daily (n=11)	6.00±3.44	85.9±3.70	-0.79±0.87	19.8±3.26	19.9±3.26	92.6±2.70	83.1±3.84	1.15±0.74	25.6±3.47	25.7±3.47	87.6±1.71
	Twice daily and more (n=6)	4.50±5.09	83.3±5.07	-0.60±1.22	21.0±3.96	21.0±4.00	90.9±3.74	82.7±1.88	1.27±0.43	26.9±1.98	26.9±1.98	87.3±1.01
Tongue-brushing habit	Yes (n=95)	5.33±3.92	85.6±3.80	-0.64±1.09	20.0±3.82	20.1±3.80	92.3±3.48	83.1±4.00	1.29±0.52	26.6±3.33	26.6±3.34	87.3±1.09
	No (n=45)	4.64±3.76	85.6±4.39	-0.51±1.24	20.2±4.02	20.2±3.97	92.2±4.27	82.8±3.77	1.30±0.58	25.8±3.48	25.8±3.50	87.2±1.14
Beverage habits	Cola (n=41)	5.71±3.80	86.3±4.36	-0.47±1.21	20.7±4.27	20.8±4.26	91.8±3.71	84.0±3.58	1.29±0.47	26.8±3.27	26.8±3.26	87.3±0.95
	Buttermilk (n=57)	4.25±3.89	86.0±3.35	-0.91±1.05	19.3±3.94	19.3±3.89	93.3±3.77	82.7±3.88	1.39±0.48	26.3±3.31	26.3±3.33	87.0±0.89
	Juice (n=25)	6.96±3.71	84.2±4.67	-0.22±1.23	20.8±3.10	20.9±3.08	91.1±3.49	82.7±4.88	1.24±0.67	26.5±3.81	26.6±3.83	87.4±1.41
	Coffee (n=5)	3.60±2.61	83.0±4.26	-1.12±1.20	17.7±4.30	17.7±4.24	94.5±4.68	81.9±3.70	1.04±0.65	25.0±3.28	25.1±3.30	87.7±1.50
	Tea (n=8)	3.38±3.42	85.5±3.28	0.02±0.64	21.3±2.41	21.3±2.41	90.1±1.87	82.4±2.05	1.18±0.27	25.5±2.74	25.6±2.77	87.3±0.53
	Water (n=4)	5.00±3.56	83.8±3.07	-0.32±0.74	21.2±3.12	21.2±3.12	91.1±1.95	80.5±3.57	0.87±1.1	23.4±4.12	23.5±4.11	88.1±2.75
Dietary habits	Protein (n=65)	4.97±3.78	85.3±3.93	-0.57±1.22	19.8±4.04	19.9±3.99	92.4±4.24	82.7±4.21	1.27±0.63	26.3±3.64	26.3±3.65	87.3±1.28
	Carbohydrates (n=56)	5.13±3.95	86.2±4.21	-0.55±1.11	20.4±3.90	20.5±3.88	92.1±3.32	83.5±3.95	1.29±0.46	26.4±3.52	26.4±3.52	87.2±0.95
	Vegetables (n=7)	6.43±5.44	84.5±2.25	-0.70±0.68	20.0±3.35	20.0±3.37	91.2±2.72	82.0±2.70	1.31±0.51	25.9±1.69	26.0±1.68	87.1±1.04
	Fruit (n=12)	5.00±3.28	85.0±4.04	-0.86±1.11	19.7±3.35	19.7±3.32	93.0±3.28	82.8±2.47	1.40±0.35	26.5±2.00	26.5±2.07	87.0±0.76
Sugar in tea	None (n=68)	5.35±3.64	85.7±4.16	-0.85±1.08	19.3±3.67	19.4±3.61	93.1±3.77	83.1±4.09	1.26±0.57	25.9±3.21	25.9±3.21	87.3±1.32
	One teaspoon (n=23)	4.96±4.73	86.0±3.91	-0.33±1.06	20.5±3.81	20.6±3.81	91.4±3.17	83.3±3.59	1.21±0.63	25.8±4.00	25.8±4.03	87.5±1.08
	Two teaspoons (n=27)	5.22±3.67	85.6±3.96	-0.64±1.16	20.0±4.07	20.0±4.02	92.3±4.12	83.0±4.58	1.38±0.45	27.3±3.72	27.3±3.74	87.1±0.68
	Three teaspoons and more (n=22)	4.36±3.97	84.9±3.71	-0.01±1.18	22.1±3.79	22.1±3.80	90.4±2.99	82.2±2.82	1.38±0.39	26.9±2.60	27.0±2.59	87.1±0.76

**Table 2.** Omnibus ANOVA test and p-values for linear multiple regression analysis

Parameters	DMFT	MC1					MP1				
		L*	a*	b*	Chroma	Hue	L*	a*	b*	Chroma	Hue
Age	0.228	0.770	0.583	0.251	0.274	0.403	0.430	0.922	0.052	0.053	0.289
Gender	0.733	0.663	0.286	0.923	0.902	0.314	0.128	0.861	0.999	1.000	0.926
Family income	0.056	0.847	0.668	0.463	0.470	0.655	0.221	0.051	0.051	0.051	0.088
Smoking habit	0.292	0.497	0.072	0.024*	0.022*	0.094	0.550	0.704	0.693	0.694	0.690
Tooth-brushing habit	0.076	0.400	0.047*	<0.001*	<0.001*	0.140	0.645	0.119	0.612	0.625	0.275
Dental floss habit	0.970	0.815	0.865	0.625	0.617	0.815	0.637	0.690	0.305	0.311	0.322
Mouthwash habit	0.458	0.503	0.627	0.265	0.262	0.666	0.653	0.759	0.692	0.691	0.765
Tongue-brushing habit	0.622	0.615	0.844	0.873	0.844	0.834	0.288	0.740	0.567	0.583	0.486
Beverage habits	0.062	0.147	0.058	0.324	0.339	0.025*	0.574	0.732	0.453	0.458	0.751
Dietary habits	0.763	0.633	0.946	0.969	0.968	0.501	0.941	0.735	0.967	0.970	0.638
Sugar in tea	0.777	0.849	0.317	0.342	0.362	0.266	0.241	0.779	0.793	0.786	0.921

Type 3 sum of squares, \* p<0.05.



**Figure 1.** Correlation matrix plots for DMFT vs. L\*, a\*, and b\* values of MC1 and MP1



**Figure 2.** Correlation matrix boxes: green and orange boxes indicate positive and negative correlations, respectively (color tones indicate the strength of the correlation). Boxes without “X” indicate significance (p<0.05); numbers on boxes are Spearman correlation coefficients

b\*, chroma, or hue in the linear regression analysis (p>0.05) (Tables 1 and 2).

The DMFT index was positively significantly correlated with MC1 a\*, b\*, and chroma values and negatively significantly

correlated with hue values (p<0.05). No significant correlation was found between L\* values and DMFT scores (p>0.05). For the MP1, no significant correlation was detected between DMFT scores and other parameters (p>0.05) (Figures 1 and 2).

**Discussion**

Several studies have shown that permanent anterior teeth correlate with each other in terms of color [8, 9]. Based on these findings, Lee [8] stated that the color of other teeth can be estimated by referring to the color of a single tooth. As a carious lesion will create a demineralized area in the tooth resulting in an inaccurate shade measurement, only caries-free teeth were used in the present study. Since the presence of multiple carious teeth in the oral cavity will restrict the shade measurement of all of the teeth, the MC1 and MP1 were selected as references for the anterior and posterior teeth, respectively. This was the major limitation of the present study.

The first null hypothesis of this work, which was that the color of the MC1 is not a predictor of caries burden, was rejected. A positive correlation was found between dental caries and MC1 a\*, b\*, and chroma values. Enamel, due to its highly inorganic content, is capable of reflecting light in high amounts and this high optical density increases the light value; dentine, in contrast, contains more organic components and thus absorbs more light and increases the chroma value [6]. Alterations in the absorption amount of light due to differences in the inorganic structure and thickness of the enamel cause differences in the chroma values of teeth [10]. When the enamel rods and the surrounding interprismatic material are parallel to the dentin layer, light can easily reach the dentine, which increases the chroma value [11]. Higher amounts of organic matter in the tooth structure can also increase both hypomineralization and the chroma value [12]. Various studies have found an association between hypomineralization and dental caries [13]. However, no significant correlation was found between the L\* value of the MC1 and total number of dental caries. Eimar et al. [14] stated that hydroxyapatite crystal size is a major predictor of light values. They found that the differences in hydroxyapatite crystal size do not change the light values while causing a change in chroma. They also stated that the main factors causing differences in chroma are the lattice parameters of hydroxyapatites, such as the a-axis and c-axis, and the lengths

of these parameters are inversely related to chroma [14]. The second null hypothesis was accepted; there was no statistical difference between the MP1 chroma and light values and the total dental caries burden. Enamel thicknesses and crystal structures of the MC1 and MP1 differ from each other [15, 16]. Oguro et al. [10] found that variations in enamel thickness cause differences in tooth color. Differences in both enamel thickness and crystal structures may limit the ability of the MP1 to predict total dental caries burden [17, 18]. In the present study, it was shown that tooth-brushing and smoking habits affected the chroma values of the MC1. Some previous studies found that cigarette smoke can decrease  $L^*$  and increase  $a^*$  and  $b^*$  values, consistent with the findings of the present study [19]. Tooth-brushing can also provide beneficial outcomes in terms of reducing cigarette stains, although not as much as tooth whitening [20]. In addition, in many studies, associations between habits of brushing and beverages and dental caries have been shown [21, 22]. The probable reason for the lack of an association between dental caries and any confounding factor in the present study may be that the subject group was narrow and restricted, which was a serious limitation of this study. The fact that the age range of the subjects was narrow and that their socioeconomic statuses and habits were similar may have raised the threshold of the occurrence of statistical difference.

Vieira-Junior et al. [23] found that as the roughness of the tooth surface increased,  $L^*$  decreased and  $a^*$  increased. Accumulation of plaque on dental tissues can increase the roughness of the tooth surface and disrupt the ideal crystal structure in the enamel over a long period of time [24]. In the present study, although polishing of the tooth surface was performed before measuring the color, any plaque that remained on the tooth surface for a long time may have impaired the optical properties of the tooth. The same is true for smoking habits. Polishing may not be sufficient to remove the deep discoloration of teeth caused by cigarettes and additional treatments such as whitening may be required to completely eliminate the discoloration of the teeth [25].

This study indicated that some color factors of the MC1 correlated with dental caries burden, and clinicians can use the color parameters of the MC1 as indicators to predict total dental caries burden. However, a common confounding factor affecting both dental caries and tooth color was not observed in this study. There may be different factors not evaluated in this study that affect both dental caries and tooth color, such as enamel and dentin thickness and composition. In this context, in future studies, it should be investigated whether the structural factors of the tooth have a common effect on the correlation of dental caries and tooth color.

### Conclusion

Both tooth color and dental caries have multifactorial etiologies, and brushing, dietary habits, and morphological characteristics of the teeth can also have an influence. Dental caries burden may be predicted based on the chroma,  $a^*$ , and  $b^*$  values of the MC1. However, light value alone is insufficient to predict the dental caries burden. This study found no significant association between color and caries burden when evaluating the MP1. The ability to predict dental caries by evaluating the

color of the MC1 shows the potential for color to be used as a part of caries risk assessment. However, further studies with wider demographics are required to support these hypotheses.

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### Scientific Responsibility Statement

The authors declare that they are responsible for the article's scientific content including study design, data collection, analysis and interpretation, writing, some of the main line, or all of the preparation and scientific review of the contents and approval of the final version of the article.

### Animal and human rights statement

All procedures performed in this study were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. No animal or human studies were carried out by the authors for this article.

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### Conflict of interest

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