# Dental biofilm in children with normal weight, overweight and obesity: a pilot study

María F Regalado Guerrero<sup>1</sup>, Liliana Iñiguez Gutiérrez<sup>2</sup>, Juan R Gómez Sandoval<sup>3</sup>, Inocencia G Ramírez López<sup>4</sup>, Gloria Y Gutiérrez Silerio<sup>5</sup>, José M Chávez Maciel<sup>1</sup>, Celia Guerrero Velázquez<sup>3</sup>, Ruth Rodríguez Montaño<sup>3,6</sup>

- 1. Universidad de Guadalajara, Centro Universitario de Ciencias de la Salud, Especialidad de Odontopediatría, Departamento de Clínicas Odontológicas Integrales, Guadalajara, Jalisco, México.
- 2. Departamento Académico de Ciencias Básicas, Universidad Áutónoma de Guadalajara, Zapopan, México.
- 3. Universidad de Guadalajara, Instituto de Investigación en Odontología, Centro Universitario de Ciencias de la Salud, Departamento de Clínicas Odontológicas Integrales, Jalisco, México.
- 4. Universidad de Guadalajara, Centro Universitario de los Valles, Departamento de Ciencias de La Salud Ameca, Jalisco, México.
- 5. Universidad Autónoma De Querétaro, Facultad De Medicina, Laboratorio De Endocrinología Y Nutrición, Centro De Investigación Biomédica Avanzada, Querétaro, México.
- 6. Universidad de Guadalajara, Centro Universitario de Tlajomulco de Zúñiga, Departamento de Salud-Enfermedad como Proceso Individual y Colectivo, Jalisco, México.

#### **ABSTRACT**

Microorganisms attached to surfaces form intricate colonies known as biofilms. Dental plaque is the biofilm formed on the tooth surface, including the gingival sulcus. Plaque staining makes it easier to see which areas need more brushing time, and which have higher risk of periodontal disease or caries. Dental plaque is largely influenced by diet, becoming more and more dependent on dietrelated carbohydrates as it ages. Inadequate dental care and bad eating habits are frequently associated with the presence of visible bacterial plaque. Aim: The aim of this study is to evaluate the percentage of dental plaque according to body mass index (BMI) and to identify whether the plaque is newly deposited, mature or acidified. Materials and Method: Twenty-five patients from the Pediatric Dentistry Clinics at the University of Guadalajara were evaluated. The percentage and type of plaque were identified using a disclosing gel. The percentage of plaque was compared among children with normal weight, overweight and obesity. Results: 14 children were normal weight, six were overweight, four were obese, and one was underweight. Average percentage of total plaque was 70.92%. The most predominant plaque was newly deposited (pink staining), followed by mature plaque (purple staining), and a lower percentage of acidified plaque (light blue staining). Conclusion: The high percentage of plaque indicates lack or inefficacy of tooth brushing, highlighting the importance of using plaque disclosure for diagnostic and educational purposes for children and parents. Keywords: dental plaque - normal weight - overweight - obesity

# Placa bacteriana dental en niños con normopeso, sobrepeso y obesidad: un estudio piloto

### RESUMEN

Los microorganismos adheridos a las superficies forman colonias intrincadas conocidas como biopelículas. La placa dentobacteriana es la biopelícula formada sobre la superficie del diente incluso en el surco gingival. La tinción con placa hace que sea más fácil ver qué áreas necesitan más tiempo de cepillado y qué áreas tienen un mayor riesgo de enfermedad periodontal o caries. La placa dental se acumula en los dientes y está influenciada en gran medida por la dieta. La placa se vuelve cada vez más dependiente de los carbohidratos relacionados con la dieta a medida que envejece. Además, un cuidado dental inadecuado y malos hábitos alimentarios se asocian frecuentemente con la presencia de placa bacteriana visible. Objetivo: Este estudio tiene como objetivo evaluar el porcentaje de placa dental según el índice de masa corporal (IMC) e identificar el tipo de placa presente, ya sea placa recién depositada, madura o acidificada. Materiales y Métodos: Se evaluaron veinticinco pacientes de las Clínicas de Odontología Pediátrica de la Universidad de Guadalajara. Se identificó el porcentaje y tipo de placa mediante un gel revelador. Se comparó el porcentaje de placa entre niños con peso normal, sobrepeso y obesidad. Resultados: 14 niños presentaron peso normal, seis tenían sobrepeso, cuatro eran obesos y sólo un niño tenía bajo peso. Un porcentaje medio de placa total del 70,92%. La placa que más predominó fue la placa de reciente depósito (tinción rosa), seguida de la placa madura (tinción violeta) y en menor porcentaje la placa acidificada (tinción azul claro). Conclusión: El alto porcentaje de placa indica falta de cepillado dental o ineficacia del cepillado si se practica, destacando la importancia de utilizar la divulgación de placa con fines diagnósticos y educativos para niños y padres.

Palabras clave: placa bacteriana - normopeso - sobrepeso - obesidad

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#### **Corresponding Author:**

Ruth Rodríguez Montaño ruth.rodriguez@academicos.udg.mx

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#### INTRODUCTION

Biofilms are complex communities of microorganisms adhered to surfaces. A community of microbial cells embedded in an extracellular matrix forms dental plaque, a biofilm that grows at the interface between two phases of matter, such as the tooth surface and saliva or gingival crevicular fluid (GCF)<sup>1-3</sup>.

Plaque staining helps identify areas with higher caries or periodontal risk and areas requiring more brushing time. It is one of the best strategies for teaching tooth brushing, refining brushing techniques, and establishing this habit<sup>4</sup>.

A study that examined plaque staining with gentian purple in children aged 6 to 48 months (4 years) reported findings with practical implications<sup>5</sup>. Dental plaque was highest on the primary molars, followed by the anterior teeth. These results, which are consistent with previous studies using plaque-disclosing tablets, suggest that these methods can effectively identify areas requiring more brushing time<sup>6,7</sup>.

Diet and eating habits significantly affect dental plaque and related conditions such as dental caries and periodontal diseases<sup>8</sup>. As plaque matures, it relies on dietary carbohydrates for sustenance<sup>9</sup>. The presence of visible bacterial plaque is often linked to poor nutritional habits and inadequate oral hygiene practices<sup>10</sup>.

Children with a high BMI are more likely to consume excess sugars in food such as sweets, grains, and processed/refined cereals<sup>11</sup>. In turn, sugar consumption has generally been associated with reduced diet quality, increased energy consumption and body weight gain<sup>12</sup>.

The rate of childhood overweight and obesity in Mexico is currently 17.5% according to the WHO 2022. According to ENSANUT 2020-2022, overweight in schoolchildren was 19.2%, and obesity 18.1% <sup>13</sup>.

BMI is calculated based on a person's weight and height, and children are considered normal weight, overweight or obese for their age according to reference tables<sup>14</sup>. Waist circumference is also used as an indicator to assess abdominal fat and the risk associated with metabolic diseases, providing a more complete view of the child's physical condition.

Thus, diet, weight and plaque are related. Plaque is linked to the occurrence of caries and periodontal disease<sup>15</sup>, and is thus an indicator of oral health. It

is therefore important to evaluate whether there is an association between percentages of dental plaque and BMI.

A disclosing gel that stains dental plaque pink, purple or light blue according to its age and acidogenicity is commercially available. Only one study has employed this gel to identify plaque in patients with or without asthma and their caries risk based on plaque acidogenicity<sup>16</sup>.

Dental plaque index and caries progression improve with educational reinforcement<sup>17</sup>. Proper tooth brushing and use of a plaque-disclosing dentifrice can reduce biofilm within a week.

The aims of this study were thus to determine differences in dental plaque percentage according to dentition type, body mass index and diet by evaluating the total dental plaque index and the percentages of pink (recently deposited plaque), purple (plaque with 24 hours' deposition), or light blue (acidic plaque with more than 48 hours' deposition), and to identify areas with higher cariogenic and periodontal pathogenic risk.

## MATERIALS AND METHOD

A cross-sectional study was conducted at the Pediatric Dentistry Specialty Clinic of the University of Guadalajara. Pediatric patients were invited to participate, and the objective of the study was explained to them and their parents. Once they agreed to participate, written informed consent according to the Helsinki Treaty of 2013 was obtained from all children and their parents or guardians. This study was reviewed and approved by the Bioethics and Research Committees of the University of Guadalajara under approval number CI-05323.

This study evaluated 25 children aged 4 to 10 years who visited the Pediatric Dentistry Clinic of the University of Guadalajara.

#### Selection criteria

Inclusion criteria: Patients who visited the Pediatric Dentistry Specialty Clinic at the University Center for Health Sciences, University of Guadalajara. Patients with normal weight, overweight or obesity. Patients aged 4 to 10 years. Exclusion criteria: Patients with any systemic disease or syndrome. Patients with orthopedic or orthodontic appliances. Patients with dental organs affected by any pathology

(caries, trauma, periodontal disease, amelogenesis, dentinogenesis). Patients with allergic reactions to the plaque-disclosing gel during the pre-reactivity test. Withdrawal: Patients who decide to withdraw from the study during the evaluation process.

### Identification of dental plaque

A pediatric dentist recorded the clinical history. The following aspects were evaluated: type of dentition (deciduous, mixed, permanent), molar relationship type (Class I, II or III), canine relationship, brushing frequency, dental plaque index, body mass index (BMI), type of diet, weight, height, and waist circumference.

Dental plaque was stained using the Tri Plaque Gel GC Three-Tone Plaque Disclosing Gel [CG, Tokyo Japan]. The Tri Plaque Gel was placed in a dispensing tray and applied to all dental surfaces using a swab. Subsequently, the patient was asked to rinse their mouth gently with water.

The observed results following plaque staining were interpreted as follows: Pink or red plaque on the tooth surface indicates recent plaque accumulation. Purple indicates mature plaque that has been present for at least 48 hours. Light blue indicates mature plaque with more than 48 hours' deposition and strong acid production.

After staining the plaque, all patients, accompanied by their parents or guardians, were shown the staining using a mirror, and each color was explained to them. After recording all plaque-stained areas and their respective colors, both the child and the parent or guardian were instructed on how to perform proper oral hygiene. They were provided with a toothbrush with soft, smooth bristles and toothpaste.

#### Evaluation of the dental plaque index

To assess the total dental plaque, the number of surfaces per tooth in the mouth and the number of surfaces with stained plaque were recorded to calculate the percentage.

number of stained tooth surfaces \* 100 total number of dental surfaces = total staining %

Similarly, the number of surfaces stained with each color (pink, purple or light blue) was counted, and the percentage of each color was calculated relative to the total number of surfaces.

```
number of stained tooth surfaces
with a color * 100

total number of dental surfaces
\frac{dental plaque \text{ of one}}{color \%}
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It should be noted that the amount of plaque per surface was not evaluated; only surfaces stained with any of the three colors were counted. Therefore, the sum of the percentages of each color may exceed 100%, as a dental surface may have all three plaque colors distributed on it.

### Anthropometric and dietary assessment

A nutritionist assessed BMI, waist circumference, and dietary intake. BMI was based on the child's sex, age, height and weight. The growth charts from the Centers for Disease Control and Prevention (CDC) were used to classify BMI as underweight, normal weight, overweight, or obesity.

Body weight was measured using the OMRON® HBF-514C body composition monitor [OMRON HEALTHCARE, INC., Illinois, U.S.A]. Height was measured with the Seca 213 portable stadiometer [Seca, Hamburg], and waist circumference was assessed using the SECA 201 anthropometric measuring tape for waist circumference [Seca, Hamburg].

Children's diet was evaluated using the validated instrument "Food Estimation and Consumption Scale in Children" <sup>18</sup>, which identifies the frequency of foods in children's diets. The frequency of consumption per week of different foods was evaluated, including bread, sweets, sugary drinks such as black or clear sodas, fruits, vegetables, seeds and red meat. The diet was classified as healthy, fairly healthy or unhealthy.

### Statistical analysis

Statistical analysis was conducted using SPSS v.25. Normality was assessed using the Shapiro-Wilk test, showing normality in the various variables. Subsequently, the Kruskal-Wallis and Mann-Whitney U test were applied to identify differences in the percentage of dental plaque according to dentition type, body mass index and nutritional status. A  $X_2$  test was performed to analyze the frequencies of food consumption in all study groups and the type of nutrition. A Spearman correlation test was conducted to evaluate the relationship between age, BMI, and the percentage of new (pink), mature (purple), and acidified (light blue) plaque. A p-value

 $\leq$  0.05 was considered significant.

#### RESULTS

# Identification of dental plaque

The study included 25 patients who visited the Pediatric Dentistry specialty clinic for dental care. Most of the participants were male. Average age was 6.68 years. Seven patients had temporary dentition, 16 had mixed dentition, and 2 had permanent dentition. The predominant molar and canine classes were class I. There were 17 patients with molar class I, four with molar class II, and four with molar class III. Canine class I predominated with 17 patients, and eight patients had canine class II. No significant difference was observed among the study groups for these variables (Table 1).

# Anthropometric evaluation and frequency of food consumption

Mean weight was 26.42 kg, and significantly higher in the obese group than in the normal

weight group. Mean height was 121 cm, with no significant difference among study groups. Mean waist circumference was 60.32 cm, and significantly higher in the obese group compared to the normal weight group. Mean BMI was 17 kg/m². One patient was underweight, 14 patients were evaluated as normal weight, 6 as overweight, and 4 as obese. Significant differences in BMI were observed among all study groups (Table 2).

The frequency of food consumption was evaluated weekly by six questions about food groups. The most frequently consumed foods per week were fruits, bread, cookies, juices, clear soft drinks, and seeds. The most frequently consumed foods per day were vegetables and fruits. In Table 3, the first row of each food group shows the frequency of consumption per week in the underweight, normal weight, and obesity groups. A significant difference is only observed in the frequency of consumption of clear soft drinks between the normal-weight and overweight groups, with normal-weight children

Table 1. Genera	Table 1. General data and dentition type												
	Total	Underweight n=1	Normal Weight n= 14	Overweight n= 6	Obesity n= 4	P							
Age (years)	6.68 <u>+</u> 2.15	11	6.29 ± 2.12	7.17 ± 2.31	$6.25 \pm 0.95$	P= 0.249							
Gender M/F	13/12	0/1	8 (57)/ 6 (43)	4 (67)/ 2 (33)	1 (25)/ 3 (75)	P= 0.429							
Dentition type T/M/P n (%)	7 (28)/ 16 (64)/ 2 (8)	Permanent	5 (36)/ 9 (64)/ 0 (0)	1/ (17)/ 4 (66)/ 1 (17)	1 (25)/ 3 (75)/ 0 (0)	P= 0.061							
Molar Class I/II/ III n (%)	17 (68)/ 4 (16)/ 4 (16)	I	10 (72)/ 3 (21)/ 1 (7)	3 (50)/ 1 (17)/2 (33)	3 (75)/ 1 (25)/ 0 (0)	P=0.749							
Canine Class I/	17 (68)/ 8 (32)/ 0 (0)	ı	9 (64)/ (36) 5/ 0 (0)	3 (50)/ 6 (50)	3 (75)/ 1 (25)/ 0 (0)	P=0.824							

Age data are shown as mean and standard deviation; the other variables are shown with the sample size by subgroup and (percentage). Gender M (male); Gender F (Female); Dentition T (Temporary); Dentition M (mixed); Dentition P (permanent); P (Statistical significance). A chi-square ( $\chi^2$ ) test was performed for qualitative variables, and a Mann–Whitney U test was used to analyze age. A p-value  $\leq$  0.050 was considered statistically significant.

Table 2. Anthro	Table 2. Anthropometric evaluation													
	Total	Underweight n=1	Normal Weight n= 14	Overweight n= 6	Obesity n= 4	N vs Ov	N vs Ob	Ov vs Ob						
Weight (Kg)	26.42 ± 10.40	32,8	21.43 ± 5.73	28.83 ± 10.10	38.65 ± 14.57	P= 0.099	P= 0.011	P= 0.201						
Height (cm)	121 ± 0.15	151	117 ± 14	123 ± 16	124 ± 11	P= 0.364	P= 0.288	P= 1.000						
Waist circumference (cm)	60.32 ± 10.3	59	55.79 ± 5.02	63.17 ± 9.72	72.25 ± 16.74	P= 0.096	P= 0.019	P= 0.517						
ВМІ	17.44 ± 3.99	14,3	15.33 ± 1.18	18.36 ± 2.27	24.2 ± 5.15	P= 0.006	P= 0.003	P= 0.019						

Data are shown as means and standard deviation. Kg (Kilograms); cm (Centimeters); BMI (Body Mass Index). N (Normal Weight); Ov (Overweight); Ob (Obesity); P (Statistical significance), and n (sample size). A Mann–Whitney U test was used to analyze age. A p-value ≤ 0.050 was considered statistically significant.

		Total	Underweight n=1	Normal Weight	Overweight n= 6	Obesity n= 4	N vs S	N vs OB	S vs OB
	_		n=1	n= 14					
	X	$2.72 \pm 2.62$	- (400)	$2.92 \pm 2.78$	$2.66 \pm 2.33$	$2.75 \pm 3.090$	0.893	0.822	0.823
	Never n (%)  1 per week	3 (12)	1 (100)	1 (7.1)	0 (0)	1 (25)		χ²	
Bread, cookies, and	n (%)	11 (44)	0 (0)	7 (50)	3 (50)	1 (25)			
Sweets	3 per week n (%)	5 (20)	0 (0)	2 (14.3)	2 (33.3)	1 (25)		P= 0.345	
	Every day n (%)	6 (24)	0 (0)	4 (28.6)	1 (16.7)	1 (25)			
	x	2 <u>+</u> 2.48	-	$1.78 \pm 2.42$	$2.83 \pm 3.43$	1.25 ± 1.25	0.829	0.955	0.82
	Never n (%)	9 (35)	0 (0)	5 (35.7)	3 (50)	1 (25)		χ²	
Black Soft Drinks	1 per week n (%)	7 (29)	0 (0)	5 (35.7)	0 (0)	2 (50)			
Dilliks	3 per week n (%)	5 (20)	1 (100)	2 (14.39	1 (16.7)	1 (25)		P= 0.463	
	Every day n (%)	4 (16)	0 (0)	2 (14.3)	2 (33.3)	0 (0)			
	x	$0.96 \pm 1.51$	-	1.14 ± 1.74	$0.16 \pm 0.40$	1 ± 1.41	0.029	0.766	0.23
	Never n (%)	11 (44)	0 (0)	4 (28.6)	5 (83.3)	2 (50)		$\chi^2$	
Juices and clear Soft	1 per week n (%)	11 (44)	0 (0)	9 (64.3)	1 (16.7)	1 (25)			
Drinks	3 per week n (%)	2 (8)	1 (100)	0 (0)	0 (0)	1 (25)			
	Every day n (%)	1 (4)	0 (0)	1 (7.1)	0 (0)	0 (0)			
	x	$4.16 \pm 2.62$	-	$3.71 \pm 2.75$	5 ± 2.19	$4.25 \pm 3.40$	0.289	0.780	0.72
	Never n (%)	3 (12)	0 (0)	2 (14.3)	0 (0)	1 (25)		χ²	
Vegetables	1 per week n (%)	2 (8)	0 (0)	2 (14.3)	0 (0)	0 (0)			
	3 per week n (%)	9 (36)	0 (0)	5 (35.7)	3 (50)	I (25)		P= 0.884	
Vegetables	Every day n (%)	11 (44)	1 (100)	5 (35.7)	3 (50)	2 (50)			
	x	$4.64 \pm 2.62$	<del>-</del>	4.42 ± 2.76	5 ± 2.19	$4.25 \pm 3.40$	0.590	0.863	0.72
	Never n (%)	1 (4)	0 (0)	0 (0)	0 (0)	1 (25)		χ²	
Fruits	1 per week n (%)	4 (16)	0 (0)	4 (28.6)	0 (0)	0 (0)			
	3 per week n (%)	7 (28)	0 (0)	3 (21.4)	3 (50)	1 (25)		P= 0.294	
	Every day n (%)	13 (52)	1 (100)	7 (50)	3 (50)	2 (50)			
	χ	0.96 ± 1.51	-	0.85 ± 1.02	1.5 ± 2.73	$0.75 \pm 0.5$	0.929	0.814	0.72
	Never n (%)	11 (44)	1 (100)	6 (42.9)	3 (50)	1 (25)		χ²	
Seeds	1 per week n (%)	11 (44)	0 (0)	6 (42.9)	2 (18.2)	3 (75)			
	3 per week n (%)	2 (8)	0 (0)	2 (14.3)	0 (0)	0 (0)		0,566	
	Every day n (%)	1 (4)	0 (0)	0 (0)	1 (24)	0 (0)			

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Table 3. Fred	Table 3. Frequency of food consumption per week													
	x	$2.32 \pm 1.88$	<del>-</del>	$2.64 \pm 2.23$	1.83 ± 1.32	1.75 ± 1.5	0.534	0.529	0.908					
	Never n (%)	5 (20)	0 (0)	3 (21.4)	1 (16.7)	1 (25)								
Red meat	1 per week n (%)	5 (20)	0 (0)	2 (14.3)	2 (33.3)	1 (25)								
neu meat	3 per week n (%)	13 (52)	1 (100)	7 (50)	3 (50)	12 (50)								
	Every day n (%)	2 (8)	0 (0)	2 (14.3)	0 (0)	0 (0)								

In each food section, the first row shows the mean and standard deviation of the times each food is consumed per week except in the underweight column because there is only one participant in that subgroup. The frequencies and percentages shown are the number of participants who consume each food either always, 3 times a week, once a week, or never. A chi-square ( $\chi^2$ ) test was performed for qualitative variables, and a Mann–Whitney U test was used to analyze age. A p-value  $\leq$  0.050 was considered statistically significant.

consuming more. Chi squared analysis of the distribution of consumption of all students according to BMI showed no significant difference (Table 3). According to the results of the dietary evaluation, 11 children consumed healthy foods: 2 obese, 3 overweight, 5 normal weight, and the participant with malnutrition. Thirteen had a fairly healthy diet: 9 normal weight, 2 overweight, and 2 obese. Only one of the participants in the overweight group had an unhealthy diet. No significant difference was observed (Table 4).

# Prevalence of dental plaque in children with normal weight, overweight and obesity

More than 50% of the tooth surfaces presented dental plaque, and the percentage of dental plaque was 70.92%. No significant difference was observed among percentages of total plaque according to BMI. However, a trend was observed where the percentage of total plaque was 73.49% in children with normal weight, which was higher than the 70.82% recorded in overweight children.

Regarding type of plaque, the most predominant was stained pink, with 36.29%, followed by purple, with 30.89%, and light blue, with 14.12%. Children with normal weight had significantly more newly-formed plaque (pink, 35.72%) than acidified plaque (light

blue, 11.76%) and higher prevalence of mature plaque (purple, 32.95%) than acidified plaque (light blue).

Overweight children had more newly-formed plaque (pink) than mature plaque (purple), but did not show a significant difference. Obese children had considerably more pink plaque (33.16%) than violet (26.15%) or light blue plaque (13.3%) (Table 5).

# Correlations of newly formed, mature and acidified dental plaque with body mass index

There was a positive correlation between total plaque and newly formed dental plaque (pink), but a negative correlation between mature plaque (purple) and height. A positive correlation was observed between consumption of vegetables and percentage of pink plaque, and between consumption of seeds and percentage of light blue plaque.

There were positive correlations between age and weight, age and height, age and waist circumference, weight and height, weight and waist circumference, weight and BMI, height and waist circumference, and waist circumference and BMI. Vegetable consumption was related to fruit consumption (Fig. 1).

#### **DISCUSSION**

Dental plaque is a biofilm inhabited by various oral microorganisms. Its maturation over time

Table 4. Nutrition						
Type of feeding	Total	Underweight n=1	Normal Weight n= 14	Overweight n= 6	Obesity n= 4	P
Healthy n (%)	11 (44)	1 (100)	5 (37.7)	3 (50)	2 (50)	
fairly healthy n (%)	13 (52)	0 (0)	9 (62.3)	2 (33.3)	2 (50)	0.480
Unhealthy n (%)	1 (4)	0 (0)	0 (0)	1 (16.7)	0 (0)	

The data are shown using the sample size by subgroup and (percentage). n= sample size according to whether or not the type of diet of children with normal weight, overweight, obese, and the participant with underweight is healthy or not. A chi-square ( $\chi^2$ ) test was performed. A p-value  $\leq$  0.050 was considered statistically significant.

Table 5. Dental	Table 5. Dental plaque													
	Total	Underweight n=1	Normal Weight n= 14	Overweight n= 6	Obesity n= 4	N vs S	N vs OB	S vs OB						
% Total plaque	70.92 ± 15.80	32	73.49 ± 17.04	70.82 ± 10.44	71.80 ± 4.52	<i>P</i> = 0.386	<i>P</i> = 0.425	<i>P</i> = 0.831						
% Pink plaque	36.29 ± 16.95	34	35.72 ± 19.86	40.07 ± 13.20	$33.16 \pm 15.92$	<i>P</i> = 0.283	<i>P</i> = 0.957	<i>P</i> = 0.240						
% Purple plaque	30.89 ± 23.38	39	32.95 ± 20.88	27.9 ± 36.65	26.15 ± 12.49	P= 0.479	<i>P</i> = 0.456	P= 0.829						
% Light blue plaque	14.12 ± 10.54	6	11.76 ± 9.14	21.52 ± 13.92	13.30 ± 6.71	P= 0.114	P= 0.873	P= 0.286						
Pink vs Purple	P= 0.299	-	P= 0.818	P= 0.259	P= 0.248									
Pink vs Light Blue	P= 0.001	-	P= 0.001	P= 0.065	P= 0.029									
Purple vs Light Blue	P= 0.006	-	P= 0.003	P= 0.935	P= 0.110									

The data are shown as the average and standard deviation of the percentage of plaque according to its color (state of maturation and acidification) in the groups of children with normal weight, overweight, and obesity. Regarding the participant who is underweight, the exact value is shown. A Mann–Whitney U test was used to analyze age. A p-value ≤ 0.050 was considered statistically significant.

	% Total Plaque	% Pink Plaque	% Purple Plaque	% Light Blue Plaque	Age	Weight	Height	Waist circumferenc e	BMI	Bread, Cookies, and Sweets	Black Soft Drinks	Juices and clear Soft Drinks	Vegetables	Fruits	Seeds	Red meats
% Total Plaque	1	0.446*	0.292	0.066	-0.087	-0.206	-0.159	-0.034	-0.023	0.123	-0.163	-0.15	0.186	0.148	0.047	-0.227
P		0.026	0.156	0.755	0.678	0.323	0.447	0.872	0.912	0.557	0.436	0.474	0.372	0.48	0.823	0.275
% Pink Plaque		1	-0.248	0.134	0.307	0.128	0.247	0.223	0.048	0.12	-0.015	0.132	0.440+	0.016	0.033	0.046
P			0.232	0.524	0.135	0.542	0.234	0.285	0.821	0.569	0.945	0.528	0.028	0.939	0.874	0.825
% Purple Plaque			1	-0.314	-0.383	-0.352	-0.410*	-0.346	-0.026	0.003	0.282	-0.007	0.091	0.029	-0.248	0.171
P				0.127	0.059	0.084	0.042	0.09	0.901	0.99	0.172	0.973	0.664	0.891	0.231	0.413
% Light Blue Plaque				1	-0.171	-0.121	-0.149	-0.061	0.119	-0.087	-0.256	-0.356	0.076	-0.049	0.526**	-0.349
P					0.414	0.566	0.476	0.772	0.572	0.679	0.217	0.081	0.717	0.817	0.007	0.087
Age					1	0.788**	0.930**	0.608**	0.11	-0.158	0.126	0.063	0.211	0.037	-0.072	0.05
P						0.001	0.001	0.001	0.6	0.45	0.547	0.764	0.312	0.859	0.732	0.814
Weight						1	0.887**	0.801**	0.557**	-0.19	0.097	-0.038	0.112	-0.043	-0.082	-0.025
P							0.001	0.001	0.004	0.363	0.644	0.855	0.595	0.839	0.697	0.906
Height							1	0.661**	0.158	-0.107	0.222	0.039	0.128	0.026	-0.104	0.637
P								0	0.451	0.612	0.285	0.852	0.543	0.901	0.62	0.06
Waist circumference								1	0.604**	-0.21	0.04	-0.017	0.052	-0.048	0.046	0.775
P									0.001	0.313	0.849	0.937	0.804	0.818	0.829	-0.199
BMI									1	-0.102	-0.141	-0.225	0.113	-0.136	-0.028	0.34
P										0.628	0.501	0.279	0.59	0.515	0.893	0.272
Bread, Cookies, and Sweets										1	0.334	-0.088	0.164	0.366	-0.152	0.188
P											0.103	0.675	0.434	0.072	0.469	0.45
Black Soft Drinks											1	-0.003	-0.072	-0.013	-0.271	0.024
P												0.988	0.732	0.95	0.189	0.334
Juices and clear Soft Drinks												1	-0.129	-0.323	0.006	0.102
P													0.538	0.115	0.975	0.196
Vegetables													1	0.456+	-0.129	0.347
P														0.022	0.538	0.15
Fruits														1	-0.185	0.475
P															0.376	-0.215
Seeds															1	0.301
Red meats																1

Fig 1: Spearman correlation. The value of each correlation is shown with its p value. BMI (Body Mass Index); P (statistical significance); \* (P < 0.05); \*\* (P < 0.001).

and excessive accumulation can lead to dysbiosis, resulting in dental caries, gingivitis, periodontitis, and other diseases<sup>2,19</sup>. However, plaque can be removed by effective tooth brushing techniques, flossing, and other adjuncts, thereby preventing such diseases.

In the current study, no significant difference in the percentage of total plaque was observed among the three types of dentition. Recently formed plaque (stained pink) predominated in all children,

indicating infrequent or absent tooth brushing, since even a less effective brushing technique will remove some recently deposited plaque (less than 24 hours old). Areas of acidified plaque (stained light blue) suggest infrequent and ineffective brushing that allows plaque to mature and become acidified. It has been reported that the most predominant bacterial species in acidified plaque is *S. mutans*, and that children with more plaque of this type have more caries<sup>20</sup>.

The fact that in normal-weight children there was a significant predominance of newly-formed plaque compared to mature and acidified plaque indicates that they did not brush before their appointment.

Overweight children had more pink plaque, although the difference was not significant. They also presented the most acidified plaque, indicating low or no brushing efficiency in the last 48 hours. Obese children presented differences between pink and light blue plaque, showing a similar pattern to children with normal weight.

A relationship between the number of cavities and BMI has been reported in adolescents<sup>21</sup> underweight, overweight or obese. Dental caries was diagnosed according to the criteria recommended by the World Health Organization (WHO. Although a metaanalysis published by Angelopoulou et al. found that the results of the studies included were inconsistent. it suggests that overweight or obese children have a higher risk of early childhood cavities<sup>22</sup>. Other studies have also concluded that obese or overweight children may be more likely to have cavities<sup>23,24</sup> if they do not practice good oral hygiene discipline. High BMI and obesity have been found to be associated with the plaque index, which indicates dental plaque and periodontal pocket depth (PD), a metric strongly related to periodontal inflammation and infection<sup>25</sup>.

The connection between dental cavities and BMI is biologically plausible because dental plaque accumulation and irregular dietary habits influence the development of both dental decay and obesity<sup>26</sup>. Moreover, caries and obesity share common modifiable factors such diet and lifestyle, highlighting their interconnected nature<sup>27</sup>. Dental caries prevalence has also been related to disadvantaged socioeconomic status<sup>28</sup>.

The positive correlation between immature (pink) and total plaque suggests collinearity since there was more pink than purple and light blue plaque. In this sense, it was expected to find a positive correlation with the most prevalent type of plaque, which was pink and the height; however, this correlation was observed with the purple plaque.

The multiple correlations among anthropometric measurements show collinearity; thus, height and age are correlated. The oldest children presented the most significant amount of mature plaque, suggesting that children tend to have less hygiene as their age increases, and highlighting the importance

of implementing hygiene education strategies that effectively reduce plaque<sup>29,30</sup>. The highest amount of plaque was observed on upper and lower anterior teeth, in agreement with several other studies<sup>4,5,17</sup>. It has been observed that people who brush their teeth with their right hand have more plaque in the right upper quadrant<sup>31</sup>, perhaps because brushing is generally started more energetically in the left quadrant, and becomes less effective when it moves to the right quadrant.

Two participants had permanent dentition. One of them was found to have an early tooth replacement process since he was just ten years old, whereas permanent teeth are usually complete between 11 and 12 years of age<sup>32</sup>. It is worth mentioning that this participant was overweight according to BMI, and it has been reported that permanent dentition is acquired earlier in obese children than in children with normal weight<sup>33</sup>. Similarly, one study reported an association between nutritional status and chronology in the first molar, lateral incisor and first lower premolar in children 8, 9 and 10 years old with obesity<sup>34</sup>, and another reported that obesity and overweight were associated to early permanent tooth eruption in males<sup>35</sup>.

In contrast, the other child with permanent dentition (age 11 years) had low weight and was referred for nutritional consultation. Because he was underweight, we would have expected that he would not yet have all his permanent teeth; however, this case appears to have been an exception.

Regarding diet, healthy foods such as fruits and vegetables were consumed infrequently by most of the children. Ten of the children were overweight or obese, so although they reported that they did not frequently consume foods high in sugar, possibly when they do consume them, it is in larger quantities. The present study may provide a basis for future studies evaluating the relationship between diet, anthropometric data, percentage of dental plaque, and presence of caries and gingivitis.

It demonstrates the importance of performing dental plaque disclosure for diagnostic and educational purposes for both children and parents, which, if applied consistently, would help reduce the prevalence of caries and periodontal disease.

#### CONCLUSION

Newly formed plaque (pink) was predominant in all children, regardless of dentition type. A higher prevalence of newly formed plaque was observed in older children. Based on body mass index (BMI), no significant difference in the amount of plaque was found among children of normal weight, overweight and obese. However, the results indicate that obese children have more acidic plaque accumulation than normal-weight children, suggesting that obesity is a factor related to diet and oral hygiene. Although the results do not indicate that dental plaque accumulation depends on BMI, they demonstrate high levels of plaque in all participants. It is therefore important to teach brushing techniques and use dental plaque staining as a clinical activity, which, if applied consistently, could reduce plaque and caries rates.

#### CONFLICT OF INTERESTS

The authors declare no potential conflicts of interest regarding the research, authorship, and/or publication of this article.

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