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Correlation Between Biofilm Maturity Level and Its Influence on the Presence of Dental Caries

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Abstract | **Original Research Article**

Dental caries is the most prevalent chronic oral disease in childhood, and dental biofilm accumulation and maturity are considered major risk factors for its development. ***Objective:*** To determine the association between dental caries and the accumulation of acidogenic biofilm identified using GC Tri Plaque ID Gel®. ***Methods:*** A cross-sectional study was conducted with a statistically determined sample of 60 children aged 3–12 years attending the Pediatric Dentistry Clinic of the Division of Postgraduate Studies and Research (DEPeI), Faculty of Dentistry, National Autonomous University of Mexico (UNAM), for their first dental visit. Participants were selected according to predefined inclusion criteria. Sociodemographic data and information on current and past caries experience were collected using the dmft and DMFT indices. Acidogenic biofilm accumulation was assessed using GC Tri Plaque ID Gel® and quantified with the O'Leary Plaque Control Record. ***Results:*** A statistically significant positive correlation was found between the dmft index and the O'Leary Plaque Control Record ($p = 0.003$). In contrast, no statistically significant correlation was observed between the DMFT index and the O'Leary index ($p = 0.380$). ***Conclusions:*** No overall association was found between dental caries and acidogenic biofilm accumulation in the study population. However, when analyses were performed separately by age group, significant associations were observed in specific subgroups, suggesting that the relationship between acidogenic biofilm and dental caries may vary according to age.

Keywords: Dental Caries; Dental Plaque; Biofilms; Child.

Over time, inadequate or deficient oral hygiene in children has been recognized as a major public health problem that contributes to the development of various oral diseases, including dental caries, [1] the most prevalent chronic disease of childhood. When left untreated, dental caries can lead to pain, speech impairment, facial cellulitis, and other oral health complications. [2] (Figure 1)

Figure 1: Patient with odontogenic cellulitis

The World Health Organization (WHO) reports that the global prevalence of dental caries among children ranges from 60% to 90%. Early Childhood Caries (ECC) is a multifactorial disease that affects the primary dentition of infants and preschool-aged children. Furthermore, ECC represents a major global public health problem because of its high prevalence, severity, and long-term consequences. It is particularly common in developing countries and among vulnerable populations, including rural communities, migrants, marginalized ethnic groups, and socioeconomically disadvantaged populations in developed countries. [3] (Figure 2)

Figure 2: Early Childhood Caries (ECC)

In Mexico, updated national and regional epidemiological data on dental caries remain limited, making it difficult to accurately estimate its prevalence and incidence. According to the most recent report published in 2024 by the Epidemiological Surveillance System for Oral Diseases (SIVEPAB), 72.6% of the pediatric population was affected by Severe Early Childhood Caries (S-ECC). [4]

Dental caries is considered a multifactorial disease that affects children across different age groups and is associated with biological, sociocultural, and behavioral factors. However, evidence indicates that one of the main predisposing factors is the fermentation of dietary carbohydrates by cariogenic bacteria, particularly Streptococcus mutans, Streptococcus sobrinus, Streptococcus mitis, and Streptococcus salivarius, which are components of the dental biofilm. [4,5]

Dental caries has traditionally been assessed using the DMFT (Decayed, Missing, and Filled Teeth) index for permanent dentition, originally proposed by Klein and Palmer in 1937. In 1944, Gruebbel adapted this index for primary dentition, resulting in the dmft index, in which the "missing" component was replaced by teeth indicated for extraction because of caries, since it is often difficult to determine whether a primary tooth was lost due to dental caries or natural exfoliation. [5,6]

For an individual, the DMFT and dmft scores are calculated by summing the number of decayed, missing (or indicated for extraction in the case of primary dentition), and filled teeth. At the population level, the mean DMFT and/or dmft score is obtained by calculating the average of the individual scores. [6]

From a microbiological perspective, Streptococcus mutans, Streptococcus sobrinus, Streptococcus mitis, and Streptococcus salivarius have been recognized as important microorganisms involved in the etiology of dental caries. [4,5] Their participation within the dental biofilm plays a key role in the establishment and maintenance of the oral microbiota and in the development of an acidogenic biofilm. [1,8,9]

Dental biofilm is continuously exposed to saliva and the natural self-cleansing mechanisms of the oral cavity. However, although these mechanisms can remove food debris, they are not sufficient to effectively eliminate dental biofilm.[10]

One of the most important measures for preventing the accumulation of dental biofilm on tooth surfaces is effective toothbrushing performed using proper oral hygiene techniques. In addition, professional dental prophylaxis plays a key role in biofilm control and is supported by various methods for detecting the presence of dental biofilm. [10,11] (Figure 3)

Figure 3: Dental biofilm accumulation

Dental biofilm has a color similar to that of tooth surfaces, making it difficult to detect visually unless it is stained with a disclosing agent, which provides contrast between the biofilm and the dental surfaces. [12]

The most commonly used indices for assessing the presence of dental biofilm include the Löe and Silness Plaque Index, the Simplified Oral Hygiene Index proposed by Greene and Vermillion, and the O'Leary Plaque Control Record, introduced in 1972 by Timothy J. O'Leary. The latter evaluates four smooth surfaces of all teeth present in the oral cavity, allowing patients to identify, during each dental visit, the surfaces where biofilm remains and to perform daily self-monitoring. [13]

To record this index, a biofilm disclosing agent is required. The smooth surfaces of each tooth are evaluated at the mesial, distal, buccal, and lingual or palatal sites. The score is calculated by determining the proportion or percentage of stained surfaces in relation to the total number of examined surfaces.

Recent studies suggest that an O'Leary Plaque Control Record score equal to or lower than 20% indicates adequate oral hygiene; therefore, patients are considered to have a lower risk of developing dental caries. [13,14]

A disclosing agent is a product that stains dental biofilm, making it visible and thereby facilitating its detection and removal.[15]

As described by Cohen *et al.,* (1972), "Different formulations and presentations of biofilm disclosing agents are available, including chewable tablets, drops, gels, and mouthrinses, all of which have a similar mechanism of action." [15,16](Figure 4)

Figure 4: Dental biofilm staining with a disclosing tablet

Among these methods, a simple approach for detecting dental biofilm is the use of GC Tri Plaque ID Gel®, which allows the assessment of biofilm maturity and the identification of biofilm characteristics associated with dental caries risk. [13]

Initially, the diagnostic method available for this purpose was the first-generation GC Plaque Check + pH® fermentation test. [15] Subsequently, the second-generation GC Tri Plaque ID Gel® was developed. This method is based on the same principle of acid production; however, it allows the simultaneous evaluation of biofilm at different sites rather than performing the assessment in separate steps. The principle of GC Tri Plaque ID Gel® is based on the pH response of different dyes incorporated into a disclosing gel containing glucose, allowing the visualization of both biofilm maturity and acid production after two minutes. [17]

GC Tri Plaque ID Gel® stains biofilm light blue when sucrose is metabolized by acidogenic bacteria, indicating a pH below 5.0 and a potentially cariogenic environment. Dark blue or purple staining indicates mature biofilm with an estimated age of 24–48 hours, whereas red or pink staining represents newly formed biofilm or biofilm with a maturity of less than 24 hours. [18,19] (Figure 5)

Figure 5: Colorimetric assessment using GC Tri Plaque ID Gel®

Objective

To determine the correlation between the presence of carious lesions and the accumulation of acidogenic biofilm identified using GC Tri Plaque ID Gel® in patients attending the Pediatric Dentistry Clinic of the Division of Postgraduate Studies and Research (DEPeI), Faculty of Dentistry, National Autonomous University of Mexico (UNAM).

MATERIALS AND METHODS

A cross-sectional descriptive study was conducted involving a sample of 60 patients. The sample size was calculated using statistical probability. After sample calculation, participants were distributed into age groups and by sex using convenience sampling.

Participants were divided into three age groups (3–5.9, 6–8.9, and 9–12 years), with a sex distribution of 50% females and 50% males. The inclusion criteria were: patients attending the Pediatric Dentistry Clinic of the Division of Postgraduate Studies and Research, Faculty of Dentistry, National Autonomous University of Mexico (UNAM), for the first time; patients aged between 3 and 12 years; patients with a valid clinical record; and those whose parents or legal guardians had previously signed informed consent.

The exclusion criteria were: patients who met the inclusion criteria but presented any disability; patients younger than 3 years or older than 12 years; and patients attending follow-up appointments at the Pediatric Dentistry Clinic of the Division of Postgraduate Studies and Research, Faculty of Dentistry, UNAM.

Ethical Considerations

This study was reviewed and approved by the Ethics and Research Committee of the Faculty of Dentistry, National Autonomous University of Mexico (UNAM), under approval number CIE/0711/11/2023.

Data Collection

A data collection instrument was designed to obtain sociodemographic information and epidemiological data regarding current and previous caries experience in permanent and primary teeth using the DMFT and dmft indices, respectively. The O'Leary Plaque Control Record was used for the identification and quantification of acidogenic biofilm. (Figure 6)

Figure 6: Data collection forms for the DMFT/dmft indices and the O'Leary Plaque Control Record.

Clinical Procedure

The study objectives and the procedures for recording the DMFT, dmft, and O'Leary Plaque Control Record indices were verbally explained to the parents or legal guardians of each participant. After all questions and concerns had been addressed, informed consent was obtained.

For the clinical examination, each patient was positioned in the dental chair in a supine position at 180°. The dental condition of each participant was initially assessed using the diagnostic criteria of the DMFT and dmft indices. Clinical examination was performed using the dental unit light and a No. 5 mouth mirror. All tooth surfaces were evaluated after being dried with air from the triple syringe. For the purposes of this study, teeth classified as "filled with recurrent caries" were also considered as "decayed."

Figure 7

Subsequently, the biofilm disclosing agent (GC Tri Plaque ID Gel®) was applied to all tooth surfaces using a microbrush. The patient was instructed to distribute the disclosing agent throughout all tooth surfaces using the tongue. The gel was allowed to act for two minutes, according to the manufacturer's instructions, to ensure adequate staining and enable the assessment of biofilm maturity.

After the waiting period, excess dye was removed with water without pressure, and the remaining water was removed using a saliva ejector. The O'Leary Plaque Control Record was then completed, recording only the tooth surfaces showing accumulation of acidogenic biofilm (turquoise-blue staining). (Figure 8)

Figure 8: Application of GC Tri Plaque ID Gel® for dental biofilm assessment

The examiner was calibrated for the epidemiological assessment of the indices by a reference examiner from the Department of Preventive Dentistry and Public Health of the Faculty of Dentistry, National Autonomous University of Mexico (UNAM). The following agreement rates were obtained: 93% agreement for the DMFT index (p = .000) and 87% agreement for the dmft index (p = .000). For the O'Leary Plaque Control Record, the agreement rate was 90% (p = .000).

RESULTS

Descriptive analysis

A total of 60 patients were evaluated (n = 60). The mean age was 7.2 years (SD ± 2.67). As shown in Table 1, 50% of the participants were female and 50% were male (Table 2).

Table 1: Age distribution of the study population

Mean	N	SD
7.20	60	2.679

Table 2: Distribution of patients according to sex

		Frequency	Percentage	Valid percentage	Cumulative percentage
Valid	1 (female)	30	50.0	50.0	50.0
	2 (male)	30	50.0	50.0	100.0
	Total	60	100.0	100.0	

The sample was divided into three age groups: Group 1: 3–5.9 years, Group 2: 6–8.9 years, and Group 3: 9–12 years (Table 3).

Table 3: Age distribution of the study population

		Frequency	Percentage	Valid percentage	Cumulative percentage
Valid	1 (3-5.9 yrs)	20	33.3	33.3	33.3
	2 (6-8.9 yrs)	20	33.3	33.3	66.7
	3 (9-12 yrs)	20	33.3	33.3	100.0
	Total	60	100.0	100.0	

Group distribution of oral health indicators

The overall mean dmft score was 8.22, while the overall mean DMFT score was 2.48. The mean O'Leary Plaque Control Record score was 57.47% (Table 4).

Table 4: Overall mean scores of the dmft, DMFT, and O'Leary Plaque Control Record indices.

	ceo	CPO	O´Leary
Mean	8.22	2.48	57.47
N	50	40	60
SD	5.227	3.178	26.121

When the distribution of the dmft, DMFT, and O'Leary Plaque Control Record scores was analyzed according to age group, the following results were obtained:

Group 1: The mean dmft score was 12.20, and the mean O'Leary Plaque Control Record score was 56.05% (Table 5).

Table 5: Mean dmft and O'Leary Plaque Control Record scores in Group 1

	CEO	O´Leary
Mean	12.20	56.05
N	20	20
SD	5.095	29.876

The mean dmft score among female patients in Group 1 was 11.4, and the mean O'Leary Plaque Control Record score was 42.20% (Table 6).

Table 6: Mean dmft and O'Leary Plaque Control Record scores by sex: Female, Group 1

	ceo	O´Leary
Mean	11.4	42.20
N	10	10
SD	5.016	23.351

In male patients from the same group, the mean dmft score was 13.0, and the mean O'Leary Plaque Control Record score was 69.9% (Table 7; Figure 9).

Table 7: Mean dmft and O'Leary Plaque Control Record scores in male patients from Group 1

	ceo	O´leary
Mean	13.00	69.90

N	10	10
SD	5.312	30.212

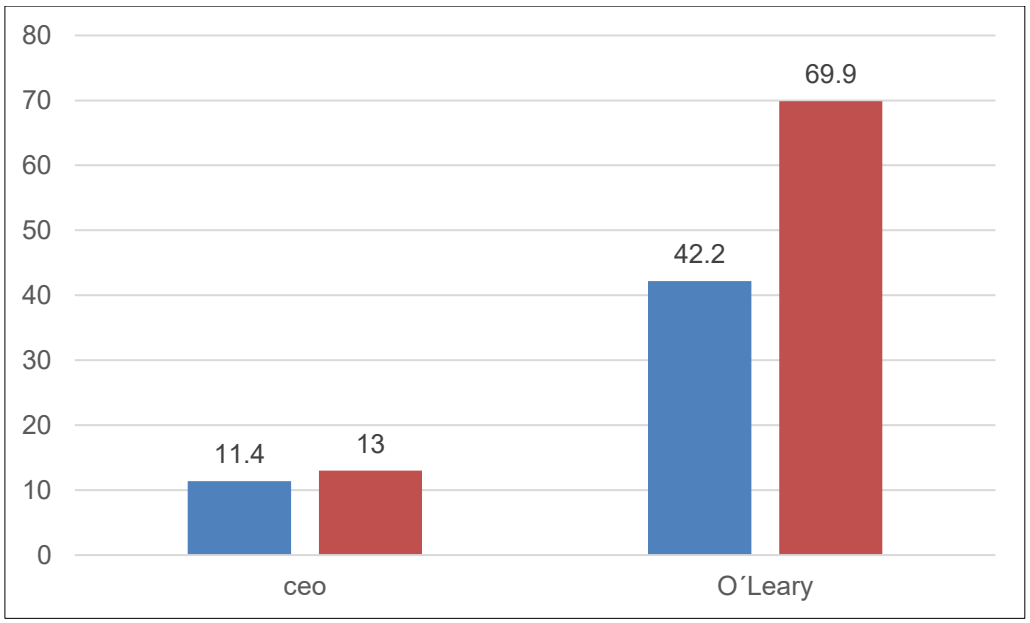

Figure 7: Mean dmft and O'Leary Plaque Control Record scores in Group 1

Group 2: The mean dmft score was 6.40, whereas the mean DMFT score was 0.90. The mean O'Leary Plaque Control Record score was 53.05% (Table 8).

Table 8: Promedio de índices ceo, CPO y O´Leary. Grupo 2

	ceo	CPO	O´leary
Mean	6.40	0.90	53.05
N	20	20	20
SD	3.378	1.294	26.154

The mean dmft score among female patients in Group 2 was 6.10, the mean DMFT score was 0.90, and the mean O'Leary Plaque Control Record score was 52.40% (Table 9).

Table 9: Mean dmft, DMFT, and O'Leary Plaque Control Record scores in female patients from Group 2

	ceo	CPO	O´Leary
Mean	6.10	0.90	52.40
N	10	10	10
SD	3.446	1.524	24.869

In male patients from Group 2, the mean dmft score was 6.70, the mean DMFT score was 0.90, and the mean O'Leary Plaque Control Record score was 53.70% (Table 10; Figure 10).

Table 10: Mean dmft, DMFT, and O'Leary Plaque Control Record scores in male patients from Group 2

	ceo	CPO	O´leary
Mean	6.70	0.90	53.70
N	10	10	10
SD	3.466	1.101	28.717

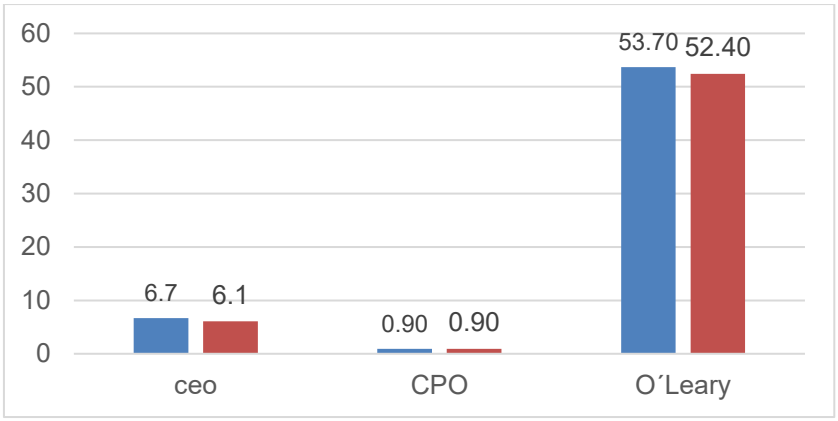

Figure 10: Mean dmft, DMFT, and O'Leary Plaque Control Record scores in Group 2

Group 3: The mean dmft score was 3.90, while the mean DMFT score was 4.05. The mean O'Leary Plaque Control Record score was 63.30% (Table 11).

Table 11: Mean dmft, DMFT, and O'Leary Plaque Control Record scores in Group 3

	ceo	CPO	O´Leary
Mean	3.90	4.05	63.30
N	10	20	20
SD	2.558	3.720	21.991

In female patients, the mean dmft score was 3.00, the mean DMFT score was 3.30, and the mean O'Leary Plaque Control Record score was 59.10% (Table 12).

Table 12: Mean dmft, DMFT, and O'Leary Plaque Control Record scores in female patients from Group 3.

	ceo	CPO	O´Leary
Mean	3.00	3.30	59.10
N	5	10	10
SD	2.449	3.401	19.140

In male patients, the mean dmft score was 4.80, the mean DMFT score was 4.80, and the mean O'Leary Plaque Control Record score was 67.50% (Table 13; Figure 11).

Table 13: Mean dmft, DMFT, and O'Leary Plaque Control Record scores in male patients from Group 3.

	ceo	CPO	O´Leary
Mean	4.80	4.80	67.50
N	5	10	10
SD	2.588	4.050	24.807

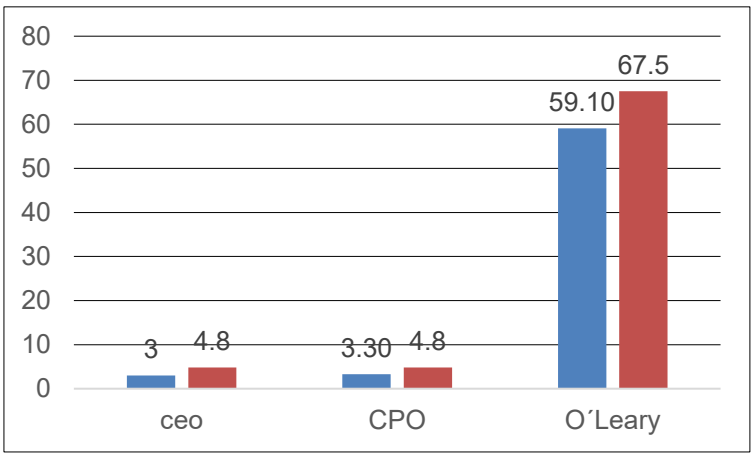

Figure 11: Mean dmft, DMFT, and O'Leary Plaque Control Record scores in Group 3

When analyzing the distribution of dmft, DMFT, and O'Leary Plaque Control Record scores according to sex, the following results were obtained:

Female sex: The mean dmft score was 7.60, the mean DMFT score was 2.10, and the mean O'Leary Plaque Control Record score was 51.23% (Table 14; Figure 12).

Table 14: Mean dmft, DMFT, and O'Leary Plaque Control Record scores in female patients

	ceo	CPO	O´leary
Mean	7.60	2.10	51.23
N	25	20	30
SD	5.123	2.845	22.909

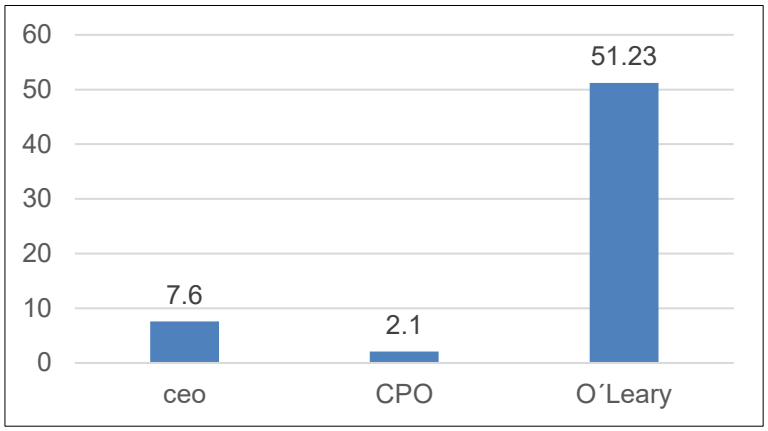

Figure 12: Mean dmft, DMFT, and O'Leary Plaque Control Record scores in female patients

Male sex: The mean dmft score was 8.84, the mean DMFT score was 2.85, while the mean O'Leary Plaque Control Record score was 63.70% (Table 15; Figure 13).

Table 15: Mean dmft, DMFT, and O'Leary Plaque Control Record scores in male patients

	ceo	CPO	O´Leary
Mean	8.84	2.85	63.70
N	25	20	30
SD	5.359	3.514	27.980

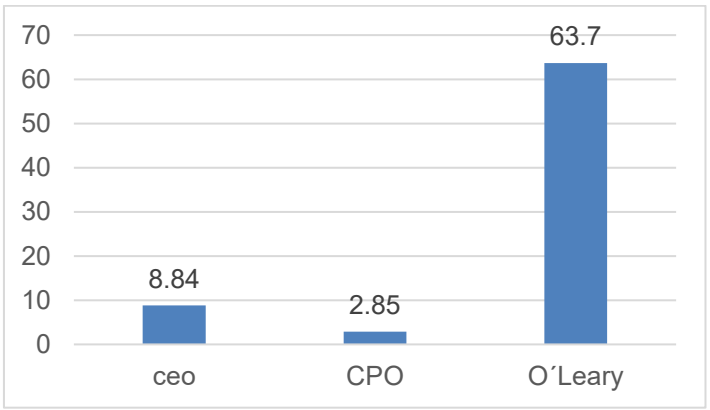

Figure 13: Mean dmft, DMFT, and O'Leary Plaque Control Record scores in male patients.

The descriptive analysis of the distribution of dmft, DMFT, and O'Leary Plaque Control Record scores by age group and sex showed that, across the three analyzed groups, the dmft index exhibited similar behavior in both sexes. In age group 2 (6–8.9 years), greater current and previous caries experience was observed among female patients.

Regarding the O'Leary Plaque Control Record, as shown in Figures 1, 2, and 3, greater dental biofilm accumulation was identified among male patients in all three age groups.

Analytical analysis

The correlation analysis between the O'Leary Plaque Control Record and the dmft index showed an r value of 0.414 and p = 0.003, indicating a positive and statistically significant correlation between both indices (Table 16).

Table 16: Pearson's correlation between dmft and O'Leary Plaque Control Record scores in the total sample

		ceo	O´Leary
ceo	Pearson correlation	1	.414**
	Sig. (bilateral)		0.003
	N	50	50
O´Leary	Pearson correlation	.414**	1
	Sig. (bilateral)	0.003	
	N	50	60

However, the correlation analysis between the O'Leary Plaque Control Record and the DMFT index showed an r value of 0.143 and p = 0.380, indicating that there was no statistically significant correlation between acidogenic biofilm accumulation and the presence of dental caries in permanent dentition (Table 17).

Table 17: Pearson's correlation between DMFT and O'Leary Plaque Control Record scores in the total sample

		CPO	O´Leary
CPO	Pearson correlation	1	0.143
	Sig. (bilateral)		0.380
	N	40	40
O´Leary	Pearson correlation	0.143	1
	Sig. (bilateral)	0.380	
	N	40	60

Regarding the distribution by age group, Group 1 showed an r value of 0.654 and p = 0.002, indicating a statistically significant correlation between both indices (Table 18).

Table 18: Pearson's correlation between dmft and O'Leary Plaque Control Record scores in Group 1

		ceo	O´Leary
ceo	Pearson correlation	1	.654**
	Sig. (bilateral)		0.002
	N	20	20
O´Leary	Pearson correlation	.654**	1
	Sig. (bilateral)	0.002	
	N	20	20

In Group 2, the correlation between the dmft index and the O'Leary index yielded an r value of 0.535 and a p value of 0.015, indicating a statistically significant correlation (Table 19).

Tabla 19: Correlación entre índices ceo y O´Leary. Grupo 2

		ceo	O´LEary
ceo	Pearson correlation	1	.535*
	Sig. (bilateral)		0.015
	N	20	20
O´LEary	Pearson correlation	.535*	1
	Sig. (bilateral)	0.015	
	N	20	20

In contrast, the correlation between the DMFT index and the O'Leary index in Group 2 yielded an r value of 0.358 and a p value of 0.121, indicating that no statistically significant correlation existed between these indices (Table 20).

Table 20: Correlation between the DMFT Index and the O'Leary Plaque Index in Group 2

		CPO	O´LEary
CPO	Pearson correlation	1	0.358

	Sig. (bilateral)		0.121
	N	20	20
O´LEary	Pearson correlation	0.358	1
	Sig. (bilateral)	0.121	
	N	20	20

The correlation between the dmft index and the O'Leary index in Group 3 yielded an r value of 0.709 and a p value of 0.022, indicating a positive and statistically significant correlation between these variables (Table 21).

Table 21: Correlation between the dmft Index and the O'Leary Plaque Index in Group 3

		ceo	O´Leary
ceo	Pearson correlation	1	.709*
	Sig. (bilateral)		0.022
	N	10	10
O´Leary	Pearson correlation	.709*	1
	Sig. (bilateral)	0.022	
	N	10	20

For the same group, the correlation between the DMFT index and the O'Leary index yielded an r value of −0.078 and a p value of 0.744, indicating that no statistically significant correlation existed between these indices (Table 22).

Table 22: Correlation between the DMFT Index and the O'Leary Index. Group 3

		CPO	O´Leary
CPO	Pearson correlation	1	-0.078
	Sig. (bilateral)		0.744
	N	20	20
O´Leary	Pearson correlation	-0.078	1
	Sig. (bilateral)	0.744	
	N	20	20

DISCUSSION

According to the World Health Organization (WHO), dental caries is the most common chronic oral disease among children, with a worldwide prevalence ranging from 60% to 90% of the pediatric population.[3] In Mexico, the prevalence of severe early childhood caries is 72.6%, according to the most recent evaluation conducted by SIVEPAB in 2024.[4] The results of the present study show a prevalence of 96.66% among patients attending the Pediatric Dentistry Clinic of the DEPeI, Faculty of Dentistry, UNAM, representing an alarmingly high prevalence rate. This finding may be overestimated because the study was conducted in a dental care facility, where most patients seek treatment due to existing signs of disease (dental caries).

According to Orellana *et al.,*(2022), the WHO proposes five levels of severity based on population dmft (ceo) and DMFT (CPO) indices. Consistent with these criteria and the results of the present study, patients aged 3 to 5.9 years were classified as having severe risk. Patients in Group 2 were classified as having moderate risk according to the dmft index, whereas the DMFT index indicated a very low risk. Patients aged 9 to 12 years were classified as having moderate risk according to both indices. One possible explanation for the variation in severity levels may be the length of time that teeth have been present in the oral cavity. [3]

Orellana also concluded that dental caries prevalence was higher among male patients than among female patients, contrasting with the findings of the present study, in which both the dmft and DMFT indices showed similar patterns in both sexes. [3]

Silva *et al.,*(2008) reported that dental biofilm is composed of Streptococcus mutans, Streptococcus sobrinus, Streptococcus mitis, and Streptococcus salivarius, and that these microorganisms play a determining role in biofilm development and fermentation.[5] Likewise, Benítez *et al.,*(2017), in their article "Correlation of the DMFT and dmft Indices with the Simplified Oral Hygiene Index," stated that dental caries is a disease of multifactorial etiology associated with biological, social, cultural, and behavioral factors. However, evidence suggests that one of the principal factors is the fermentation of carbohydrates by Streptococcus mutans. [4]

Ojeda *et al.,* (2013) reported that poor oral hygiene habits are largely responsible for the accumulation of dental biofilm, its fermentation, and consequently the development of carious lesions in

primary, mixed, and permanent dentitions. [1] The results of the present study support these findings, with a mean O'Leary Plaque Index of 57.47%. The same authors also described dental caries as a dynamic process resulting from microbial metabolism occurring on the tooth surface, emphasizing that time is a determining factor because prolonged exposure ultimately leads to the development of carious lesions. Since the present study employed a cross-sectional design, the effect of time could not be evaluated; therefore, it was not possible to establish a significant correlation between biofilm accumulation and the presence of carious lesions.

CONCLUSION

The correlation analysis between the presence of dental caries lesions (assessed using the dmft and DMFT indices) and the accumulation of acidogenic dental biofilm (measured using the O'Leary Plaque Index) in the overall sample demonstrated that no significant relationship exists between these variables.

Only when the analysis was performed within specific age groups and restricted to the dmft index did the relationship tend to reach statistical significance.

Dental caries is a multifactorial disease in which time is considered one of the determining factors for its development. Therefore, longitudinal studies are required to adequately evaluate the relationship between the presence of carious lesions and the accumulation of acidogenic dental biofilm.

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