Abstract: The aim of this study was to determine the correlation between laser fluorescence (LF) values of apparently sound tooth enamel and caries history. LF measurements were recorded from six sound enamel tooth surfaces in each of 346 subjects aged 8-25 years. Caries experience was evaluated using the decayed, missing, and filled tooth (DMFT) index and the International Caries Detection and Assessment System (ICDAS). To measure differences between the unrestored and restored tooth groups, an unpaired two-sample t-test was used. To assess the relationship between LF of sound enamel and caries experience based on the DMFT and ICDAS, Pearson’s correlation coefficient and linear regression analyses were used. The LF values of sound dental enamel in subjects with no history of invasive dental treatment were highly correlated with caries experience, as measured by the DMFT index (R = 0.76) and ICDAS (R = 0.7). The LF values of sound enamel in subjects with a history of previous invasive dental treatment were weakly correlated with caries experience, as measured by the DMFT index (R = 0.33), and moderately correlated with the ICDAS values (R = 0.66). The LF value of clinically healthy tooth enamel correlates with caries status based on the DMFT or ICDAS.

Keywords: caries risk assessment; laser fluorescence; sound enamel.

Introduction

Dental caries continues to be a major health concern in many populations (1). Changes in dietary habits and lifestyle in many developed countries in recent years have been accompanied by changes in the prevalence and distribution of dental caries among populations (2). Caries tends to be concentrated in a small proportion of the population; large proportions of populations are apparently caries-free in young age groups, but over time caries-free dentition develops decay. Therefore, identification of individuals who are at high risk of caries is desirable in order to provide them with preventive care, and also to implement population-wide preventive strategies (3).

Risk assessment is an essential component in the decision-making process for prevention and contemporary management of dental caries. The search for acceptable, accurate, and cost-effective strategies for identifying high-risk individuals has intensified, and multiple risk factors and indicators have been proposed as targets. Tests for caries risk can assist prediction of its development, while clinical signs and history are equally important for assessing the main causes of caries in any given individual (4). Studies examining the predictive potential of several factors have shown that a history of caries (particularly involving the first permanent molar) continues to be the best predictor of future caries in pre-school children and schoolchildren/adolescents (5,6). However, it is not a particularly useful predictor in young children, since it is important to determine the
risk of caries before it actually develops (7), and previous caries is a “risk factor” that cannot be modified by the dentist (8). Therefore, it is important to have risk indicators that can predict the susceptibility of individuals to dental caries before lesions appear. To this end it would be desirable for dentists to detect changes in apparently sound enamel before lesions become evident.

The methods currently used by dentists to record caries history involve the detection of cavitated lesions and use of the decayed, missing, and filled tooth (DMFT) index. Additionally, the International Caries Detection and Assessment System (ICDAS) records lesions that do not involve cavitation of the enamel. Recently, additional diagnostic methods and devices for the detection of dental caries have been introduced; it is now also possible to detect non-cavitated carious lesions through the presence of bacteria and their metabolites within the enamel using laser fluorescence (LF). Carious regions emit stronger fluorescence in the red and infrared ranges of the spectrum ($\lambda = 655$ nm) than do sound/healthy regions (9); hence, the fluorescence from a carious region is greater than that from a sound/healthy region.

The origin of fluorescence in carious regions is oral bacterial porphyrins. Sound enamel generates LF values ranging from 0 to 13, while carious enamel produces values ranging from 14 to 20 (10). As sound enamel becomes carious, it develops porosity due to the ongoing action of bacterial acids, allowing bacterial by-products such as protoporphyrins and organic acids to penetrate the enamel surface. Since this occurs at the microscopic level, clinical detection at this early stage is not possible. However, the most porous lesions would be expected to demonstrate higher LF values, as more bacterial products would be able to enter these areas.

Therefore, the present study was conducted to investigate the correlation between the LF values of clinically sound enamel and caries history to establish a more reliable method of assessing caries risk. The working hypothesis was that LF values of clinically healthy enamel would correlate positively with the DMFT and ICDAS scores in patients with no history of dental treatment.

Materials and Methods

Ethical considerations
This study was approved by the research ethics committee of the Faculty of Higher Studies, Iztacala, National Autonomous University of Mexico (protocol 1014. 27-jun-2014), and was conducted in accordance with the Declaration of Helsinki.

Population and sampling
The study was conducted on a non-probabilistic sample comprising subjects aged 8-25 years. The sample size was calculated using a priori G*Power analysis with the following parameters: effect size, 0.3; $\alpha$-error probability, 0.05; power, 0.95; sample size, 134 (this number was considered the minimum) (11). The parents of 270 children aged 8-17 years received a letter describing the proposed investigation and were asked to give consent for their children to participate. Eighty subjects $\geq$18 years provided consent for clinical examinations after receiving an explanation of the procedures. Individuals were examined in accordance with the guidelines of the World Health Organization (WHO) for epidemiological studies in oral health (Oral Health Survey, Basic Methods. 5th edition. World Health Organization, Geneva, 2013 [http://www.who.int/oral_health/publications/9789241548649/en/]). Upon completion of the study, all subjects and parents were given a report of the subject’s assessed caries risk level.

The inclusion criteria were as follows: voluntary participation in risk assessment for tooth decay, and no food consumption 1 h prior to data and sample collection. Subjects with fixed orthodontic appliances or enamel development defects were excluded.

The subjects were divided into two groups based on their dental history: an unrestored tooth group having no history of surgical/invasive dental treatment, and a restored tooth group.

Standardization of the examiners
During the training process, two undergraduate examiners evaluated 19 subjects (5.49% of the sample size, not from the study population) and measured the following variables twice, at an interval of 1 week: the DMFT index, the ICDAS, and LF values. Inter-rater agreement was calculated as: DMFT, kappa = 0.733-1.00; ICDAS, kappa = 0.723-1.00; and LF, kappa = 0.829-1.00).

Measurement of the LF of sound smooth tooth surfaces
Tooth surfaces were cleaned using the modified Bass technique of brushing, with a new toothbrush and tap water; no auxiliary toothpaste and/or mouthwash was used, to avoid alterations in the degree of enamel fluorescence. Tooth surfaces were then air-dried using a triple syringe, and cotton rolls were used for isolation. LF measurements were obtained with a DIAGNOdent pen (KaVo, Biberach, Germany) using the flat-tipped probe recommended for smooth surfaces (tip B), in accordance with the manufacturer’s instructions. The measurement
time was standardised to approximately 10 s. The LF of the lesion-free surfaces of six teeth used in the Simplified Oral Hygiene Index (OHI-S) (12) was assessed by running the tip of the pen gently across the surface of each tooth from the gingival to the incisal/occlusal margin, and the reading was recorded.

Caries experience assessed in terms of the DMFT index
Dental caries in each subject was clinically evaluated under natural light by previously trained researchers, with the subject seated in a dental or regular chair. Periodontal probes, calibrated in millimetres, with rounded tips (WHO 621), and plane No.5 dental mirrors, were used. The examinations were conducted in accordance with the methodology recommended by the WHO.

Caries experience assessed in terms of the ICDAS
Next, the clean teeth were assessed in accordance with the ICDAS criteria (International Caries Detection and Assessment System Coordinating Committee [2012] Rationale and Evidence for the International Caries Detection and Assessment System [ICDAS II], https://www.iccms-web.com/uploads/asset/592848be55d87564970232.pdf. Accessed 15 July 2014), first while wet, and then after drying with compressed air for 5 s.

A dental mirror and a WHO 621 periodontal probe were used as visual-tactile aids during the surface assessments. To obtain a caries history index using the ICDAS coding, the registry values of each ICDAS code obtained from each tooth were summed. In subjects who had previously undergone invasive tooth treatment, a code was assigned according to the type of restoration and its current state, as follows: code 2, amalgam and resin restorations with sealed margins; code 3, amalgam or resin restorations with loss of marginal integrity; code 6, teeth missing due to caries; and code 0-6 for teeth without restorations.

Statistical analysis
Data were analyzed using the Statistical Package for the Social Sciences version 22.0 (SPSS Inc., Chicago, IL, USA). The distribution and frequency of study variables (DMFT, ICDAS score) were obtained. To measure differences between the subject groups, unpaired two-sample t-test was used. To assess the associations between LF and caries experience (DMFT and ICDAS), Pearson’s correlation coefficient was used; these tests were two-tailed. Linear regression analyses were performed to assess the effect of LF values on the DMFT or ICDAS code using LF values as the predictor variable and the DMFT and ICDAS as dependent variables. The results of the correlation were classified according to the method developed by Hinkle et al. (Applied Statistics for the Behavioral Sciences. Boston, Houghton Mifflin, 2003).

Results
A total of 346 subjects aged 8 to 25 years (mean 13.07 years) were recruited; 54.6% of the subjects were female and 45.4% were male. The subjects were divided into an unrestored (n = 214) or a restored (n = 132) tooth group, based on treatment history. The mean LF values (± standard deviation) in the unrestored and restored tooth groups were 5.13 (±2.99) and 6.24 (±2.23), respectively. The values registered for each assessment and group are shown in Table 1.

The unrestored tooth group had significantly lower LF values, DMFT scores, and ICDAS scores (P < 0.01) than the restored tooth group.

The method developed by Hinkle et al. revealed that, in the unrestored tooth group, the LF was strongly correlated with the DMFT and ICDAS scores (Table 2). In mouths with previously treated teeth, the LF values explained 43.2% of the variation of the caries lesions as coded with the ICDAS. LF explained only 10% of the

| Table 1 Comparison of the variables associated with dental caries between the subject groups |
| --- |
| Variables | Restored tooth group n = 214 | Unrestored tooth group n = 132 | P-value |
| Mean | SD | Mean | SD |
| Laser fluorescence | 5.13 | 2.99 | 6.24 | 2.23 | < 0.001 |
| DMFT | 0.79 | 1.72 | 4.27 | 3.02 | < 0.001 |
| ICDAS | 11.64 | 11.73 | 16.4 | 10.53 | < 0.001 |

DMFT: decayed, missing, and filled teeth; ICDAS: International Caries Detection and Assessment System; SD: standard deviation. P-values were determined using Student’s t-test. n = 346.
variation in the DMFT index.

**Discussion**

In the present study, strong correlations were observed between LF values and caries experience, as determined using the DMFT and ICDAS values. Linear regression analysis allowed an estimation of how changes in the LF values of clinically sound enamel can predict changes in the DMFT and ICDAS values in dentition without prior treatment. This analysis yielded a potentially predictive model that reflected the need for preventive treatment of healthy dental enamel, based on the association between low LF values and previous experience of caries, as evaluated using the ICDAS and DMFT indices in patients without previous dental treatment. This model may help with treatment decision-making for minimally invasive treatment of enamel surfaces without frank cavitation in patients with previous restorative dental treatment, thus diminishing the risk of overtreatment.

LF was not recorded for all tooth surfaces, and only for six surfaces in order to facilitate clinical work. These surfaces are used in the OHI-S, which is widely employed to collect information on plaque accumulation (13,14). The LF values obtained in the present study can be considered to reflect previous lesions that have become remineralized (15). According to in vivo studies of experimental caries, after 1 week of undisturbed biofilm formation, no clinical changes were observed in the enamel, even after the samples had been carefully air-dried (16). However, at the ultrastructural level, there were signs of direct dissolution of the outer surface of the enamel. This was characterized by expansion of the inter-crystalline spaces due to partial dissolution of the peripheries of individual crystals (17). These spaces can trap bacteria that are embedded in remineralized areas. The presence of microorganisms has been demonstrated in the lesions of incipient active and inactive caries using scanning electron microscopy (18). Therefore, it is possible that penetration of their metabolic products into the enamel tissue in early caries lesions produces an increase in fluorescence values, even if the enamel is clinically sound.

A history of demineralization-remineralization episodes has been described in epidemiological studies where the presence of initial caries lesions, detected in molars that had partially erupted and then became remineralized after several years, were considered clinically healthy surfaces (19). Such surfaces were evaluated in the present study.

Since LF detects bacteria or porphyrins that are already present in the enamel structure, LF values for clinically healthy enamel can be considered an indicator of both disease (i.e. submicroscopic lesions) and risk factors (i.e. bacteria associated with the caries development). The ability to use LF values to predict caries lesions is important, as some other caries risk assessment systems involving bacteriological testing are costly and time-consuming. Although LF analysis has a high initial cost, the day-to-day expenses are lower, thus making it more profitable in the long term.

Table 2 Correlations between LF and dental caries history

|                | Unrestored dentition | Restored dentition |
|----------------|----------------------|--------------------|
|                | $n = 214$            | $n = 132$          |
| DMFT           | LF   | 0.76*  | 0.33*  |
| ICDAS          | 0.70* |        | 0.66*  |

The r values are presented. DMFT: decayed, missing, and filled teeth; ICDAS: International Caries Detection and Assessment System; LF: laser fluorescence. *Correlation is significant at the $P < 0.001$ level (two-tailed).
is important to develop strategies or methods that allow reliable prediction of the future appearance of carious lesions, even in patients without a history of caries. Nevertheless, no reliable method for estimating the risk of caries in children and adolescents has been reported to date.

Some studies have suggested that preventive strategies based upon LF measurements, especially in school children from low-income families and with high caries risk, represent a cost-effective strategy that might reduce subsequent need for provision of restorative services (25).

One limitation of the present study was the wide age range of the subjects (8-25 years). Additionally, data from only 346 subjects were used; therefore, additional longitudinal studies with larger populations should be performed to confirm the predictive value of LF. Due to the strong positive correlation between LF values and caries history in terms of the ICDAS and DMFT indices, a future longitudinal cohort study should be performed to evaluate the potential benefits of LF measurement, to determine whether this is a cost-effective method for monitoring enamel surfaces and conducting preventive and interventional dental health strategies. Given that LF values show great sensitivity and more specificity than the ICDAS criteria as a diagnostic method, it has been recommended that these methods be combined to improve diagnosis (26).

In conclusion, the present study has revealed that the LF value of apparently sound dental enamel in dentition without previous invasive treatment correlates strongly with caries history as measured using the DMFT or ICDAS indices. The LF of sound enamel in dentition with invasive treatment was weakly correlated with caries experience as measured with the DMFT index, and moderately correlated with the ICDAS score. Collectively, the present data indicate that LF values for healthy tooth enamel obtained using the OHI-S may be useful for constructing reliable caries risk assessment systems.

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Conflict of interest
The authors declare that they have no conflict of interest.

References
1. Bagramian RA, Garcia-Godoy F, Volpe AR (2009) The global increase in dental caries. A pending public health crisis. Am J Dent 22, 3-8.
2. Frazão P (2012) Epidemiology of dental caries: when structure and context matter. Braz Oral Res 26, 108-114.
3. Pitts NB (1997) Diagnostic tools and measurements-impact on appropriate care. Community Dent Oral Epidemiol 25, 24-35.
4. Zero D, Fontana M, Lennon AM (2001) Clinical applications and outcomes of using indicators of risk in caries management. J Dent Educ 65, 1126-1132.
5. Messer LB (2000) Assessing caries risk in children. Aust Dent J 45, 10-16.
6. Mejäre I, Axelsson S, Dahlén G, Espelid I, Norlund A, Tranæus S et al. (2014) Caries risk assessment. A systematic review. Acta Odontol Scand 72, 81-91.
7. American Academy of Pediatric Dentistry (2013) Guideline on caries-risk assessment and management for infants, children, and adolescents. Pediatr Dent 35, E157-E164.
8. Petersson GH, Isberg PE, Twetman S (2010) Caries risk assessment in school children using a reduced Caries Index. BMC Oral Health, doi: 10.1186/1472-6831-10-5.
9. Lussi A, Hibst R, Paulus R (2004) DIAGNOdent: an optical method for caries detection. J Dent Res 83, C80-83.
10. Lussi A, Megert B, Longbottom C, Reich E, Francescut P (2001) Clinical performance of a laser fluorescence device for detection of occlusal caries lesions. Eur J Oral Sci 109, 14-19.
11. Faul F, Erdfelder E, Lang AG, Buchner A (2007) G Power 3: a flexible statistical power analysis program for the social, behavioral, and biomedical sciences. Behav Res Methods 39, 175-191.
12. Green RM, Vermillion JR (1964) The simplified oral hygiene index. J Am Dent Assoc 68, 7-13.
13. Al Hashmi H, Kowash M, Hassan A, Al Halabi M (2017) Oral health status among children with cerebral palsy in Dubai, United Arab Emirates. J Int Soc Prev Community Dent 7, S149-154.
14. Mashima I, Theodorea CF, Thaweboon B, Thaweboon S, Scannapieco FA, Nakazawa F (2017) Exploring the salivary microbiome of children stratified by the oral hygiene index. PLoS One12 e0185274.
15. Moriyama CM, Rodrigues JA, Lussi A, Diniz MB (2014) Effectiveness of fluorescence-based methods to detect in situ demineralization and remineralization on smooth surfaces. Caries Res 48, 507-514.
16. Holmen L, Thyrlstrup A, Ogaard B, Kragh F (1985) A polarized light microscopic study of progressive stages of enamel caries in vivo. Caries Res 19, 348-354.
17. Holmen L, Thyrlstrup A, Ogaard B, Kragh F (1985) A scanning electron microscopic study of progressive stages of enamel caries in vivo. Caries Res 119, 355-367.
18. Parolo CC, Maltz M (2006) Microbial contamination of noncavitated caries lesions: a scanning electron microscopic study. Caries Res 40, 536-541.
19. Backer DO (1966) Posteruptive changes in dental enamel. J Dent Res 45, 503-511.
20. Addy M (1986) Plaque control as a scientific basis for the prevention of dental caries. J Soc Med 79, 6-10.
21. Zhang Q, Bian Z, Fan M, van Palenstein Helderman WH (2007) Salivary mutans streptococci counts as indicators in caries risk assessment in 6-7-year-old Chinese children J Dent 35, 177-180.
22. Hong X, Hu DY (2010) Salivary Streptococcus mutans level: value in caries prediction for 11-12-year-old children. Community Dent Health 27, 248-252.
23. Powell LV (1998) Caries prediction: a review of the literature. Community Dent Oral Epidemiol 26, 361-371.
24. Chaffee BW, Featherstone JD, Gansky SA, Cheng J, Zhan L (2016) Caries risk assessment item importance: risk designation and caries status in children under age 6. JDR Clin Trans Res 1, 131-142.
25. Fontana M, Platt JA, Eckert GJ, González-Cabezas C, Yoder K, Zero DT et al. (2014) Monitoring of sound and carious surfaces under sealants over 44 months. J Dent Res 93, 1070-1075.
26. Iranzo-Cortés JE, Terzić S, Montiel-Company JM, Almerich-Silla JM (2017) Diagnostic validity of ICDAS and DIAGNOdent combined: an in vitro study in pre-cavitated lesions. Lasers Med Sci 32, 543-548.