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ORIGINAL ARTICLE

Interest in a new test for caries risk in adolescents undergoing orthodontic treatment

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Abstract It has been reported that patients undergoing orthodontic treatment present a high risk of caries. Recently, an immediate chair-side test was proposed, displaying the intra-oral lactic acid production of cariogenic bacteria. The aim of this 12-month follow-up prospective cohort study was to evaluate the association between having a high score on this test and caries occurrence in 110 young patients scheduled for orthodontic treatment. Caries occurrence was studied by Kaplan–Meier curves and Multivariate Cox models allowed the examination of its association with covariates. Fifty four patients developed at least one carious lesion during the follow-up period. At baseline, approximately 70% of the patients presented a high risk of caries according to the test and this number came close to 80% by the study's completion. According to the Kaplan–Meier estimator, 51% ($CI_{95\%}$ 0.40, 0.60) of the sample would have developed at least one carious lesion during the follow-up. The test score was then associated with age, DMFT, and caries occurrence. This study showed that a high test score at baseline associated with a high DMFT predicted a high risk of caries (RR=2.6). Taking the patient's age into consideration, an increase of 1 year resulted in a 10% decrease of the risk of caries occurrence (RR=0.89). Within the limits of this longitudinal study, it may be concluded that this test is useful to evaluate the risk for dental caries in adolescents with orthodontic treatment. Furthermore, the distribution of the lesions in our sample suggests specific clinical approaches for this group of patients.

Keywords Caries risk · Predictive test · Orthodontic treatment · Adolescent · Carious lesion

None of the co-authors have known conflicts of interest

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Orthodontic treatments have been reported to increase the risk of developing carious lesions [8, 24, 36]. This risk may be attributed to orthodontic appliances, which compromise plaque control and consequently lead to plaque accumulation [44]. In addition, the majority of patients undergoing orthodontic treatment are teenagers. This may enhance the risk of poor compliance regarding plaque control and prevention [25]. It has also been shown that orthodontic treatment may affect the distribution of oral biofilm,

increasing the concentration of *Streptococci mutans* [4, 31]. Furthermore, some research reported that pH in the plaque of the upper incisors is generally lower than in other parts of the bonded dentition. This is presumably due to the low clearance of saliva in this area [3].

In presence of a cariogenic environment, the enamel demineralization process around the bracket progresses rapidly [23, 34]. Therefore, once the appliances are removed, after approximately 2 years, white spots are diagnosed around brackets. Some white spot lesions may remineralize and return to a visually acceptable appearance. However, some lesions persist, resulting in an aesthetically unacceptable result. In severe cases, restorative treatment may be required. To prevent this, many approaches have been developed including improved plaque control methods, antimicrobial therapy [4], amorphous calcium phosphate incorporation in bonding materials [43], and appropriate modes of fluoride delivery [30].

Caries risk assessment before the treatment and monitoring during the follow-up is advisable. Recently, an immediate chair-side test, Clinpro Cario L-Pop (CCLP), has been proposed. This test measures the lactate production, i.e., the cariogenic activity of the dental plaque [11, 22, 38]. Cariogenic bacteria such as S*treptococci mutans* metabolize dietary sugars resulting in the production of acids [46]. The bacterial lactic acid excretion increases when sugars are available in excess, leading to dental hard tissues demineralization. In low-risk patients, buffers from saliva balance this process. However, for high-risk patients, such host barriers are overcome and frequent demineralization leads to the formation of carious lesions. The CCLP test does not determine specific bacteria in the oral cavity but reveals their main metabolic product, which is lactic acid.

The purpose of this 12-month follow-up cohort study was to evaluate the association between a high score obtained on the CCLP test and caries occurrence in patients undergoing orthodontic treatment.

Materials and methods

Study population

This was a multicenter, 12-month follow-up study. One hundred and fourteen patients were consecutively enrolled during the initial consultation in the orthodontic departments of Bretonneau and Charles Foix public hospitals in Paris (France). Recruitment was completed from November 2004 to December 2005. The study was approved by the hospital's Ethical Committee.

Patients and their parents were first given oral and written information on the study's purpose. Consent was given by signing a protocol.

To be included in the study, patients had to fulfill the following inclusion criteria:

1. Age: 10–25 years (10<age<25)
2. Absence of any active carious lesion
3. Scheduled for orthodontic treatment with mono or bi arcade, involving at least four permanent teeth per maxillary and a wire covering the entire denture for an expected duration of at least 12 months.

Patients were excluded from the study if they met any of the following exclusion criteria:

1. Active carious lesion
2. Periodontitis
3. Partial or total removable prosthesis
4. Pregnancy
5. Poor medical conditions (diabetes, asthma…), systemic antibiotics, or local antimicrobials 2 months preceding the orthodontic treatment placement, patients under current medication affecting the saliva flow rate.

During the study, a patient starting a medication affecting the saliva flow rate or the biofilm composition was immediately excluded.

Clinical examination

All included subjects were thoroughly examined before inserting the fixed appliances by an experienced clinician from the conservative dentistry department. They received both clinical and X-ray dental examinations. DMFT and DMFS were calculated from records and bitewing radiographs. The saliva flow rate and buffer were determined (CRT Buffer, Vivadent, Lichtenstein).

Study design

The study was carried out over five visits. Prior to the first visit, potential enrolled subjects were given routine dietary and oral hygiene instruction (O.H.I.) with fluoride toothpaste in the orthodontic department. During the first visit, the patients meeting the inclusion criteria were given a consent form in order to be enrolled in the study. Plaque control was checked during this appointment. O.H.I. was reinforced, and the appliance placement was postponed in subjects not having an optimal plaque control. At the second visit, orthodontic appliances were placed in experimental subjects. The CCLP test (Clinpro Cario L-Pop®, 3M ESPE, Seefeld, Germany) was performed according to manufacturer's instructions by the orthodontist in charge of the patient, and read by a senior orthodontist that was blind for the patient (baseline score). A plaque index was recorded according to Butler's scores [13]. Plaque was collected in the interproximal buccal area of maxillary

molars, and plaque samples were sent to the microbiological laboratory for analysis (Dental School Toulouse, University Paul Sabatier, France). Further study visits were carried out at 3, 6, and 12 months. During these visits, the CCLP test was performed in the orthodontic department in the same conditions as previously described and the patients were subjected to a thorough clinical examination in the conservative dentistry department. O.H.I. was reinforced if necessary. During the follow-up period, the study was considered completed for any participant diagnosed with a carious lesion. The lesion/s was/were then treated and risk management was carried out. In the most severe cases, orthodontic treatment was interrupted.

Definition of outcome criteria

The CCLP test rates the risk of dental caries according to the following scale: low (1–3), moderate (4–6), and high (7–9). This biochemical test is based on the evaluation of lactic acid production of the microflora, which is assessed by a colorimetric scale. The test was used according to the manufacturer's recommendations. Following tooth brushing, a bacterial sample was taken from the dorsum of the tongue using a stick with a cotton swab impregnated with sucrose. The dorsum of the tongue has a highly papillated surface which supports a higher bacterial density than other oral mucosal surfaces. The swab was inserted in the L-Pop blister and the blister was pressed to activate the chemical reaction, which led to a color signal within 5 min. The signal was then compared to the reference color chart provided by the manufacturer.

Ekstrand's criteria were used for caries diagnosis on occlusal and smooth surfaces at baseline, 3, 6, and 12 months [18, 20]. These criteria are based on a ranked visual scoring system (visual appearance of the lesion, plaque stagnation, tactile sensation using a ball-ended probe, bleeding of the gingiva after probing) which allows one to identify the lesion. In brief, this system relates the clinical appearance of the lesion to its severity (deepness) and its activity. According to these criteria, the diagnosis of a primary lesion is considered as positive with a score >7.

Statistical analysis

The size of the sample was calculated on the basis of the caries incidence within a year (10% for patients presenting a low risk of caries) reported by Axelsson [5, 6]. With α- and β-values set at 0.05 and 0.2, respectively, 114 subjects were needed considering that the relative risk of a high CCLP was 4, that 75% of the patients would present a high risk of caries according to the test and in anticipation of a maximum loss rate of 10% in the follow-up.

Ten patients were lost during follow-up. Therefore, the Kaplan–Meier estimator was used to evaluate the probability of caries occurrence and the multivariate Cox model was employed to assess the association between caries occurrence and covariates. Covariates were considered to be associated with caries occurrence if the corresponding p value was less than 0.05. The c-index was used to evaluate the performance of the multivariate model [26].

Results

Profile of study participants

One hundred and ten patients (mean age=15.4 years±5.2; 48.2% males) were available for analysis at the end of the 12-month follow-up period. Three patients dropped out between the inclusion and the 3-month visit, two between 3- and 6-month visit, and five between 6- and 12-month visits (Fig. 1). Five patients were lost because of relocation, one patient withdrew his informed consent, and four were excluded because of prolonged medications that might have affected biofilm composition.

Table 1 indicates the patients' characteristics at baseline. The mean age is in accordance with those of the patients following an orthodontic treatment in our dental clinics.

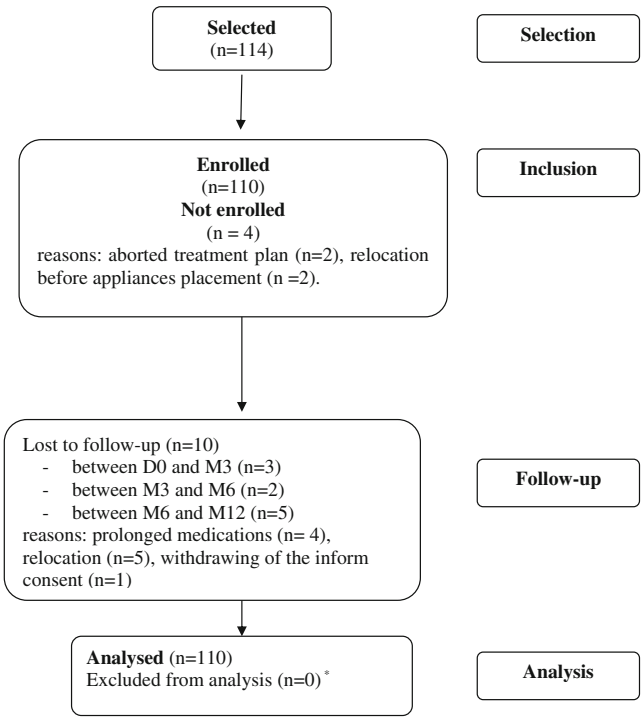

Fig. 1 Flowchart of the study with selections, inclusions, and follow-up; a total of 110 patients completed the study. *Individuals who were lost to follow-up were considered as censored in the statistical analysis

Table 1 Baseline characteristics of study population; values are expressed as mean ± standard deviation or as percentage

Variable	Statistics
Age	15.36 (5.18)
Gender	
Male	48.2%
Parental occupational status	
Small business men/Farmer	10.9%
High professions	11.8%
Intermediate and managerial professions	15.5%
Skilled worker	41.8%
Student	10.0%
Unemployed	2.7%
Others	7.3%
Tooth brushing	
Almost never	0%
Once a day	27.3%
Twice a day	53.6%
Three times a day	19.1%
More than three times a day	0%
Undetermined	0%
Consumption of soft drinks	
Almost never	30.9%
Once a day	28.2%
Twice a day	15.5%
Three times a day	11.8%
More than three times a day	13.6%
Undetermined	0%
Consumption of sweet foods between meals	
Almost never	32.7%
Once a day	35.4%
Twice a day	18.2%
Three times a day	7.3%
More than three times a day	6.4%
Undetermined	0%
DMFT[a]	2.76 (3.58)
DMFS[b]	4.47 (6.91)
Salivary flow rate	1.71 (0.74)
Buffer capacity	
Good	70.9%
Moderate	28.2%
Insufficient	0.9%
Plaque index	
Low (less than 20% of colonized surfaces)	10.9%
Moderate (between 20% and 50%)	35.5%
High (more than 50%)	53.6%
Streptococcus mutans (number of colony forming units)	
=0	0%
]0; 20]	99.1%
>20	0.9%
Lactobacillus (number of colony forming units)	
=0	0.9%
]0; 21]	92.7%
>21	6.4%

The salivary flow rate is expressed in ml/min and corresponds to a stimulated rate

[a] *DMFT* decayed/missing/filled teeth

[b] *DMFS* decayed/missing/filled surface

The sex ratio is well balanced. The parental occupational status indicates a high percentage of skilled workers, which is in accordance with the general recruitment of public dental clinic. The hygiene status of the sample indicates that the majority of the patients included in the study brush their teeth twice daily. The dietary interviews do not show any specific cariogenic behaviors with regard to the beverage and food consumption. The mean DMFT and DMFS values of the sample do not differ from those reported in national surveys [10] [32]. Similarly, the high plaque index of the sample is in accordance with the outcomes of epidemiological studies showing that teenagers have high Oral Hygiene Index [2]. Of them, 99.1% and 92.7% had between 1 and 20 CFU *Streptococcus mutans* and 1 and 21 CFU lactobacilli, respectively. This corresponds to sparsely colonized surfaces not particularly prone to new carious lesion development [7].

Caries occurrence and lesion distribution over the follow-up period

Fifty four patients developed at least one carious lesion during the follow-up period. This means that only 46 patients were not diagnosed with carious lesion at 12 months. Sixteen patients developed at least one carious lesion before the 3-month visit, 27 patients between the 3-month visit and the 6-month visit, and 11 patients between the 6-month visit and the 12-month visit, respectively. At the end of the study, the estimate caries occurrence was 0.51 ($CI_{95\%}$ 0.40, 0.60) according to the Kaplan–Meier estimator.

A total of 119 active carious lesions were diagnosed between the 3-month visit and the 12-month visit in the 54 patients that developed at least one lesion during the follow-up period. At 3 months, 36 carious lesions were diagnosed in the 16 patients that developed at least one lesion within this period (Fig. 2a). Fourteen lesions were identified either in smooth surfaces, mainly in anterior teeth and most of them were located in the enamel. At this

visit, 21 lesions were diagnosed in pits in fissures of first and second molars. Nine were strictly located in the enamel whereas 12 already involved the dentin.

At 6 months, most of the 55 lesions diagnosed in 27 patients were located in pits and fissures and mainly in molars (Fig. 2b). Only anterior teeth presented a noticeable number of lesions diagnosed on smooth faces (n=11).

At 12 months, on the 28 lesions diagnosed in a total of 11 patients, 27 were located in pits and fissures and a large number of them were located in the second (n=14) and first molars (n=8) (Fig. 2c). A total of 22 lesions were localized in the enamel.

Caries risk over the follow-up period

According to CCLP test at baseline, the percentage of subjects being in the high-risk category was close to 70%. At the end of the study, this percentage was close to 80% (Fig. 3).

Age, DMFT, and CCLP values associated with caries occurrence

We searched which covariates might be confounding factors for the study of the association between CCLP and risk of caries occurrence. Multivariate Cox models were used in conjunction with stepwise procedure. Age (RR=0.89, $CI_{95\%}$ 0.83–0.96) and DMFT at inclusion (RR=1.11, $CI_{95\%}$ 1.04–1.2) were observed to be associated with caries occurrence. A second multivariate Cox model was then built, including age, DMFT, and CCLP score to assess the predictive performance of CCLP. Under this model, age, DMFT, and CCLP score were associated with caries occurrence (Tables 2 and 3). As far as age at inclusion is concerned, an increase of 1 year resulted in a 10% decrease of the risk of caries occurrence (RR= 0.89). The interaction of DMFT and the baseline CCLP score was associated with caries occurrence, whereas neither the DMFT value alone nor the baseline CCLP score were indicative of a caries risk. A high baseline CCLP score associated with a high DMFT value such as 10, was a good risk predictor for caries development within a year (RR= 2.6) (Table 3). In contrast, a high baseline CCLP score associated with a null DMFT was not a risk predictor (RR= 0.26). DMFS also showed an interaction with CCLP similar to those reported for DMFT (data not shown). The c-index value for the complete model was 0.72 ($CI_{95\%}$ 0.65–0.79) while it was only 0.64 ($CI_{95\%}$ 0.57–0.71) for the model including age and DMFT. This result indicates an increase in discriminative power—and therefore a better predictive performance of the corresponding prognostic model—when CCLP is considered.

Discussion

The outcome of the present study shows that CCLP test is a risk predictor for caries when associated with DMFT index. We chose to test CCLP on a population of adolescents undergoing orthodontic treatment, as this population is

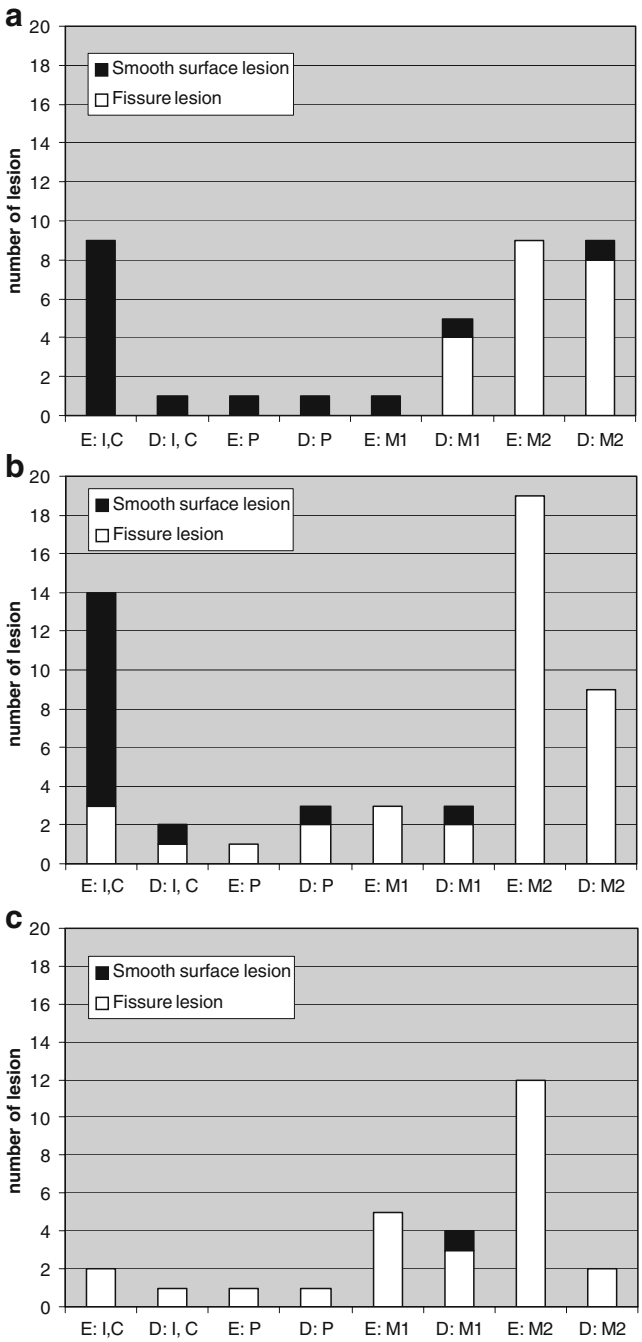

Fig. 2 Carious lesion distribution at 3 months (**a**), 6 months (**b**), and 12 months (**c**). One hundred nineteen active carious lesions were diagnosed during the follow-up period. *E* enamel lesion, *D* enamel–dentin lesion, *I* incisor, *C* canine, *P* premolar, *M* molar

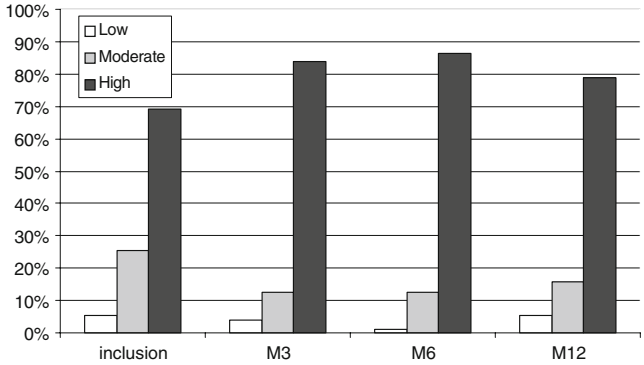

Fig. 3 Mean CCLP scores at baseline (*inclusion*), 3 months (*M3*), 6 months (*M6*), and 12 months (*M12*) of orthodontic treatment. Values are expressed in percent. One hundred and ten files were analyzed at baseline, 105 at M3, 87 at M6, and 57 at M12

particularly at risk of developing caries. In addition, clinical studies aiming to follow the incidence of dental caries in young patients usually show a high number of drop-outs [42]. In our study, the percentage of drop-outs was remarkably low, less than 10%, which might be explained by the compliance of patients with orthodontic treatment.

Our data demonstrate that the CCLP test cannot be *per se* a valuable tool for caries prediction. Tests assessing caries activity by measuring acid production by the oral bacteria were already proposed in the previous century to determine individual caries risk [21, 40]. At that time, these tests were shown not to be able to predict the risk for caries. Further studies carried out on adolescents showed that a sole test consisting in *Streptococcus mutans* count was unable to predict caries risk [29, 45]. Low bacterial scores rarely resulted in caries progression, but high S*treptococcus mutans* scores did not seem to be a sufficient parameter to predict caries incidence [41].

It has been previously shown that DMFT and more particularly DMFS were reliable parameters for caries risk prediction [17, 25, 27, 47]. In our study, we report that the combination of the test and the DMFT/DMFS index was of interest in the risk evaluation of this special patient's category. We showed that a high DMFT/DMFS index associated with a high CCLP value was a powerful predictor for lesion development.

Considering age, DMFT, CCLP value and the interaction between CCLP value and DMFT, we were able to construct a simple prognostic score which achieved an acceptable value of the *c*-index, an equivalent of the area under the ROC curve for censored data. Indeed, CCLP (along with its interaction with DMFT) increased the *c*-index from 0.64 to 0.72 and therefore improved the predictive performance of the corresponding prognostic score, even if this score should be evaluated on an external basis to be definitively validated. It is interesting to note that values between 81% and 87% of the area under the ROC curve were calculated for the prognosis of caries increment in a Dutch child population using the Swiss method of caries prognosis [42]. The difference between the outcomes of this study and ours may be due to the difference in selected variables. Nevertheless, our approach being simpler and less time consuming, it may be advisable in the routine follow-up of teenagers undergoing orthodontic treatment for a general practitioner.

High and moderate values of the test associated with caries-active infants and children have been previously reported [11]. In this latter study, nearly 75% of the patients had a high CCLP score. This is consistent with the scores we found at any given visit. It is not surprising that, according to the test, the risk of dental caries was at a high level during the study (Fig. 3). We planned to work on this orthodontic population because of the high risk of dental caries reported in the literature [8, 22, 33].

We also observed that a high CCLP value associated to a low DMFT index was not associated with caries occur-

Table 2 Relative risk estimated from the multivariate Cox model

Factor	RR	95% confidence interval for RR	
Age at inclusion	0.89	0.82	0.96
High CCLP	0.26	0.13	0.52
DMFT	0.93	0.75	1.14
High CCLP×DMFT	1.26	1.01	1.56

For *Age at inclusion* and *DMFT*, the presented relative risk corresponds to the relative risk associated to an increase of one unit: age at inclusion is expressed in years

Table 3 Relative risk of high values of CCLP (compared to low and moderate values of CCLP) as a function of DMFT

DMFT	RR for high level of CCLP	95% confidence interval for RR	
0	0.26	0.13	0.54
1	0.33	0.16	0.68
2	0.42	0.20	0.85
3	0.52	0.25	1.07
4	0.66	0.32	1.35
5	0.83	0.40	1.70
6	1.04	0.51	2.13
7	1.31	0.64	2.68
8	1.64	0.80	3.37
9	2.07	1.01	4.24
10	2.60	1.27	5.34
11	3.27	1.59	6.71
12	4.11	2.00	8.45

rence. This data could be explained by the fact that children exposed to a comparable cariogenic environment are more or less susceptible to caries [12, 15, 39]. A study aimed at the capacity of CCLP to monitor the effect of antimicrobials in the oral cavity of a population of caries-free adult patients reported that at baseline 74% of them had a high CCLP score [22]. However, one cannot compare the susceptibility to caries of newly erupted enamel to mature enamel in a cariogenic environment. It also cannot be excluded that in our patients who were initially caries-free or with a low caries experience, carious lesions might develop after a longer lapse of time. A longer period of observance might answer this question.

Age was also a relevant predictor of caries risk. We found a decrease of the caries occurrence with the increase of age at inclusion. This might be explained by the higher caries susceptibly of the newly erupted enamel, and in a cariogenic environment, active cavities may develop within a few weeks [24, 33]. Even if young patients undergoing orthodontic treatment are particularly prone to caries, we were not expecting that half of the patients developed at least one carious lesion within a year. One can suggest, but not conclude, that this incidence was directly associated to orthodontic treatment. Indeed, our study did not include a control group because it was targeted at the assessment of a test for caries risk prediction. However, this study once more highlights the need for a specific preventive approach in adolescents with orthodontic treatment, including frequent recall with oral hygiene procedure control and combination of topical fluoride, as these are the patients who should be considered as high risk [37]. There is a need for international recommendations regarding the management of caries risk in adolescents scheduled for an orthodontic treatment.

Thus, for adolescents presenting a high DMFT index associated to a high CCLP value, preventive and therapeutic measurements are required to minimize the risk of developing caries lesions and to ensure that the patient will have sound enamel structure in all teeth after completion of the orthodontic treatment. Not only must O.H.I. be given before appliance placement but also any treatment must be postponed until optimum oral hygiene achievement. In addition, oral hygiene procedures must be regularly checked during the treatment. CCLP test can be used to support O.H.I. and to monitor the effects of O.H.I. and prophylactic treatment [14, 22]. Furthermore, during the orthodontic treatment, high scores obtained from the test will conduct the clinician to reinforce individual caries risk management. Recent evidence suggests that the use of extra fluorides, in addition to fluoridated toothpaste, reduces the occurrence and severity of caries, especially white spot lesions [9, 16, 35]. Among the different topical modes of delivering fluoride, the most efficient way seems to be the daily rinsing with a 0.05% sodium fluoride mouthrinse [9]. The enamel lesions have been reported to develop on all teeth but are most frequently observed on the cervical and middle third of the buccal surface of lateral maxillary incisors, mandibular canines and first premolars [23, 24, 33–35]. The lesion distribution reported in this study is of particular interest. We chose to use visual criteria proposed by Ekstrand et al. [20], because the ICDAS II system which is the current reference for caries diagnosis was not available at the time of the study [28]. In addition, these criteria were recently shown to be concordant with ICDAS II [19]. At 3- and 6-month visits, numerous smooth surfaces lesions were diagnosed. As this type of lesions underlines a high caries risk [7], it can be suggested that the patients who developed this sort of lesion were not sufficiently prepared for orthodontic treatment. The orthodontic treatment should be postponed and these patients should first receive a better dental education regarding hygiene and dietary habits.

At 12 months, it was mainly fissures lesions that were diagnosed. Our study highlights the need for pit and fissure sealing, as recommended for permanent molars of adolescents with high caries prevalence level [1]. Furthermore, regarding our results within a year, anfractuous fissures of premolars and anterior teeth should be also sealed to prevent the colonization of these surfaces.

In conclusion, a CCLP test can be useful to monitor the caries risk during orthodontic treatment in adolescents. Associated with a past caries experience, a high score is predictive of carious lesions development within a year.

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