Oral health and obesity in the SAYCARE study: reliability and internal validity of diagnostic methods

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Summary

Objective

The aim of this pilot study was to address the reliability, internal validity and viability of oral health methods used in the South American Youth/Child cARdiovascular and Environmental study.

Methods

South American Youth/Child cARdiovascular and Environmental study was a multicentre feasibility observational study and conducted in seven South American cities. The training sessions were performed in two steps before data collection: the first verified the inter-rater reproducibility between the examiners of the six centres in relation to the gold standard, and the second one verified the inter-rater reproducibility between the examiners at each centre in relation to the main rater. The diagnostic methods used were International Caries Detection and Assessment System II and Pulpal Involvement, Ulceration, Fistula and Abscess for dental caries and Periodontal Screening and Recording and Index Plaque for periodontal disease. Anthropometric variables were measured and used to calculate the body mass index and were classified according to the cut-off points defined by the International Obesity Task Force. Cohen’s kappa coefficient and proportions of agreement were calculated to report inter-rater and intra-rater reliability in the calibration process and pilot study.

Results

The inter-rater weight kappa ranged from 0.78 to 0.88 and proportion of agreement from 96.07% to 98.10% for the International Caries Detection and Assessment System II and for the Periodontal Screening and Recording, 0.68 to 0.95 and 94.40% to 98.33%, respectively, in the calibration process. At the pilot study, a total of 490 children (40.8% overweight and 12.9% obese) and 364 adolescents (23.4% overweight and 4.3% obese) were examined. The prevalence of dental caries was 66% in children and 78% in adolescents, and gingival bleeding was 49% and 58.20%, respectively.

Conclusion

The results demonstrated good reliability and internal validity after the examiners were trained, as well as the feasibility of using the methods chosen for this multicentre study.

Keywords: Obesity, research method, youth.

Introduction

Obesity is a multifactorial chronic disease, influenced by environmental and genetic risk factors (1), and its prevalence in children has increased substantially in recent years worldwide (2). Children who are overweight or obese in adolescence are more likely to become obese adults with associated increases in morbidity such as type II diabetes, hypertension, cardiovascular disease, infectious diseases, cancer and mortality (3,4). In addition
to these general health conditions, further evidence suggests that obesity is associated with oral conditions including dental caries, periodontal disease and tooth loss (5–7). Obesity is therefore one of the primary challenges to public health, with consequences affecting many different areas of life (2).

Dental caries, as obesity, is a highly prevalent condition in childhood and adolescence with a whole-life impact. These two conditions share some common, modifiable influences such as diet and lifestyle including changes in physical activity and food preferences (8). These diseases are also associated with low family income and low level of parental education (9). Some cross-sectional studies have shown a positive correlation between caries and obesity, while others showed no effect (10,11); however, both caries and obesity are chronic conditions. The relationship between dental caries and body weight in children and adolescents has been studied in some systematic reviews (12–14), which suggested that a relationship could exist, but that is far from simplistic. Therefore, further research is recommended to assess the longitudinal relationship between obesity and caries, using a systematic approach with universal measures (12,15).

Periodontal disease is an inflammatory biofilm-dependent disease affecting the supporting tissues of the teeth, with progressive attachment loss and bone destruction (16). The effects of periodontal diseases observed in adults mostly have their initiation earlier in life (17). Indeed, the prevalence of gingivitis in children can be similar to or greater than dental caries but has received much less attention (18). The negative effect of overweight and obesity on gingivitis has been found in young subjects, mostly due to generally neglected attitudes towards oral disease prevention including self-hygiene procedures and diet (19). Additionally, obesity has been reported to be associated with periodontal disease in adults compared with non-obese individuals (20–22) because of increased levels of periodontal pathogens (23) and pro-inflammatory cytokines (24,25). Likewise, recent systematic reviews showed that the available evidence suggests a significantly positive association between periodontal disease and obesity also in children (26) and adolescents (27).

Considering the lack of longitudinal studies to understand the relationship between obesity and oral health, diagnostic methods should be reliable and validated among oral epidemiological studies. For early dental caries detection, the International Caries Detection and Assessment System (ICDAS) has proven to be reliable, offering increased sensitivity and accuracy in detecting carious lesions, and is indicated for epidemiological surveys involving children (28,29). To determine the severity and extensiveness of oral conditions that result due to untreated dental caries, the Pulpal Involvement, Ulceration, Fistula and Abscess (PUFA) index was presented and validated for epidemiological studies (30). Regarding the periodontal status, Periodontal Screening and Recording (PSR) together with Index Plaque (IP) is recommended by the American Dental Association for all dental patients as an integral part of routine oral diagnostic examinations, because the screening examination can be rapidly performed, and the results be recorded without expensive instrumentation and extensive charting (31,32).

No multicentre studies that use standardized and jointly developed methods among countries have been conducted in South America to assess lifestyle, cardiovascular health, obesity and oral health in children and adolescents. To overcome this gap in the literature, the South American Youth/Child CARdiovascular and Environmental (SAYCARE) study aims to develop methods to collect reliable, comparable and validated data about cardiovascular health biomarkers, lifestyles and environmental, social and familial factors and oral health (33). Therefore, the oral health assessment methods had to be reproducible and reliable (including the inter-rater and intra-rater reliability) considering the multicentre coverage of the study. The approach and results presented needed to be instructive to others conducting multicentre studies of oral health and obesity. Thus, the aim of this pilot study was to address the reliability, internal validity and viability of oral health methods used in SAYCARE study.

Materials and methods

Study design and sample selection

This was a multicentre feasibility observational study, entitled South American Youth/Child cARdiovascular and Environmental Study (acronym: SAYCARE study), conducted in seven South American cities (Buenos Aires/Argentina, Lima/Peru, Medellín/Colombia, Montevideo/Uruguay, Santiago/Chile, São Paulo and Teresina/Brazil) that were selected based on the presence of specialized research centres with experience in this area of research, a population of more than 500,000 inhabitants and located in different geographic areas (Figure 1).

Research subjects were selected in each city in two steps: (i) schools were selected based on the students’ age, stratified for groups (preschool [3–5 years], schoolchildren [6–10 years] and adolescents [11–17 years]) and school types (public or private; socioeconomic status proxy) on the enrolled preschool, primary school and high school and (b) random sampling was
conducted by using student lists (all pupils from a selection of classes from the selected schools). Each sex was represented by 50% of participants.

The sample size was calculated based on the experience of other multicentre projects, in which a feasibility pilot study had previously been conducted, and the reliability and validity of the used method were evaluated (34–36).

Several sample size calculations were performed in order to enable reliability analyses of the diagnostic methods and to perform the validation/agreement assessment between the two measurement methods (objective and subjective) in the study population. Each sample size calculation was adapted for each work package variable. For example, the calculation parameters used to analyse the reliability of the oral health methods were an \( \alpha \) two-tailed of 0.05 (type I error), a \( \beta \) or power (type II error) of 0.10 and a correlation coefficient and kappa coefficient of 0.80 each. From these parameters, we estimated a required sample of 28 individuals from each city for reliability/agreement analyses between the first and second measurement. Anticipating losses and rejections of 20%, we invited 240 children and 240 adolescents from each city, totalling approximately 480 research subjects. All sample size estimations are described in detail by Carvalho et al. (33).

Exclusion and inclusion criteria

The study exclusion criteria were pregnancy, the inability to complete the questionnaires and the inability of the parents, guardians and/or students to sign the informed consent form. Children who did not agree to the oral health examination after the start of the study were also excluded. The study included all subjects between 3 and 18 years old whose parents/guardians signed the informed consent form. Moreover, a signed assent form was obtained from all children and adolescents who indicated their approval to participate in the study.

Training and standardization session

The calibration aims to provide consistency to the epidemiological examinations performed by one or more examiners. For this, it is important that they adopt the same criteria during the observations, bringing down the odds to find different conclusions. On the other hand, the calculation of intra-rater agreement seeks to gauge how much the same examiner agrees with himself at different times, assessing the same patient. The study follows the method define by the World Health Organization (WHO), in which the examiner is expected to examine twice 10% of the individuals (37).
To harmonize the methodology, seven fieldwork teams (one from each city) participated in a training workshop that was held in three cities: Teresina/Brazil (March/2015), Buenos Aires/Argentina (April/2015) and Piracicaba/Brazil (August 2015), in which 2, 4 and 1 raters, respectively, participated. The raters were trained according to the WHO criteria (21) for the oral health methods and calibrated to use the ICDAS II, PUFA, PSR and IP criteria. In addition, researchers of other areas conducted anthropometric measurements (weight, height and skin-fold thickness), delivered of questionnaires and sexual maturation assessments, as described by Carvalho et al. (33).

The training was standardized in all its proceedings and consisted of both theoretical and practical sessions. The theoretical explanations of the all indexes, codes and criteria and discussion of the topics with the raters lasted 8 h. For the practical session, researchers organized a visit to a school in order to simulate the fieldwork. This included prior contact with the schools where the examinations for the training and calibration process were carried out.

In the practical discussion (12 h), each rater examined schoolchildren (7–10 years old), who were also examined by the gold standard. Children in this age group present mixed dentition, i.e., permanent and decidous teeth. The gold standard examiner (M. P. R. M.) has extensive experience with calibration programmes involving multicentre studies of oral health conditions. During this exercise, inter-rater values for these conditions were computed, and differences were compared, and discussed until a sufficient agreement between examiners (kappa coefficient) were reached. The tests were repeated until sufficient agreements between examiners (kappa coefficient) were reached (90% agreement for ICDAS II and 80% for PSR and PUFA were considered).

After this first step, the principal researcher from each city attended the workshop together with their research team. It was agreed that after this second training step, the weighted Cohen’s kappa values between the examinations of the calibration step of the researchers must be equal to or greater than 6 for the PSR/PUFA and ICDAS indexes, because this value would be acceptable to consider the evaluators as qualified for participation in the pilot study.

Clinical data collection

The exam consisted of clinical evaluation of dental caries for ICDAS II, odontogenic infection in pulp using PUFA, for periodontal conditions with PSR and PI and anthropometric parameters.

The study participants were clinically examined after the return of their informed consent form and questionnaires signed. The oral examinations were performed by dentists who had completed the calibration process, and all visual examinations were conducted under standard conditions. The examiners conducted the survey post-prophylaxis (tooth brushing if necessary) using the following: relative isolation; artificial lighting with a head-light (120 lumens) and battery replaced after every 30 tests. As it was important to evaluate both wet and dry clean teeth, the participants’ teeth were dried for 5 s with a mini compressor (200 VA, 60 Hz).

To evaluate periodontal conditions, the raters used a blunt probe Community Periodontal Index of treatment needs (CPITN), as required by the ICDAS and PSR criteria, during exams that lasted 15 min, including recording the data of each child.

The ICDAS criteria (28) were used by applying the following codes: (0) sound; (1) first visual change in enamel; (2) distinct visual change in enamel when wet; (3) localized enamel breakdown (without clinical visual signs of dentin involvement); (4) underlying dark shadow in dentin; (5) distinct cavity with visible dentin; and (6) extensive distinct cavity with visible dentin.

For the PUFA (30), the following scores were used: (S) sound; (P) visible pulpal involvement; (U) ulceration caused by dislocated tooth fragments; (F) fistula; and (A) abscess. The scores for PSR (31) were as follows: (0) sound tissue; (1) bleeding; (2) dental calculus; (3) coloured portion of the blunt probe partially visible; and (4) complete portion of the blunt probe invisible. The scores for the Silness and Loe PI (32) are as follows: (0) no plaque; (1) a film of plaque adhering to the free gingival margin and adjacent area of the tooth. The plaque may be seen in situ only after application of disclosing solution or by using the probe on the tooth surface; (2) moderate accumulation of soft deposit within the gingival pocket, or the tooth and gingival margin which could be seen with the naked eye; and (3) abundance of soft matter within the gingival pocket and/or on the tooth and gingival margin.

The anthropometric variables were analysed according to the reference manual of anthropometric standardization of the WHO (38) as follows: weight, height, waist circumference, hip circumference, neck circumference and skin-fold thickness (bicep, tricep, subcapular and suprailiac). All anthropometric variables were measured once in this order, and then, the measurements were repeated one or two more times in the same order. The third measure was performed only in case of an error of 5% between the first and the second measure. The evaluations were conducted in a private room at the school. All measures were taken in underwear or with as few clothes as possible and without shoes (33). They
were categorized as ‘normal weight’, ‘overweight’ or
‘obese’ according to World Obesity/Policy and Prevention
(International Obesity Task Force) cut-off values (38).

Intra-rater agreement was achieved by repeated
examination (with a 2-week interval between repeated mea-
asurements) in a number of individuals and the subsequent
comparison between each pair of examinations. Each rater
re-examined approximately 10% of the sample, by repeating
one individual in each group of 10 (37).

The raters were blinded with regard to the subjects’
information. The entire organization was carried out by
the Organizing Committee of SAYCARE study, for both
calibration and training and the pilot study.

Statistical analysis

Data analysis involved calculation of the Cohen’s kappa
coefficient and proportions of agreement on a tooth-to-
tooth basis during both the training and pilot study
periods, to determine intra-rater and inter-rater reliability
and agreement values. The Cohen’s weighted kappa
was chosen because all the variables were considered
ordinal; the categories were assumed to be ordered,
and accounted for how far apart the raters were (39),
and because it is the most used in oral health studies
(39–41). The frequency of data distribution was
determined in the pilot study. Descriptive statistics for
anthropometric parameters were calculated by the
International Obesity Task Force categories. The dental
caries prevalence was recorded when at least one tooth
received code 1 or higher. Scores 1 to 3 were considered
initial caries, and scores 4 to 6 were considered severe
dental caries with lesion extending through the middle
third of dentin. Statistical analysis was carried out using
the Statistical Package for Social Sciences (SPSS for
Windows, version 18.0, SPSS Inc., Chicago, IL, USA).

Ethical considerations

This study received approval from the Human Ethics
Research Committee of the Faculty of Medicine of the
University of São Paulo (research protocol no. 232/14).
All parents/guardians received information regarding the
objectives of the study and signed the term of free and
of informed consent.

Results

The examiner training process involved two steps. The
first step verified the inter-rater reproducibility of the
examiners of the six centres (São Paulo, Teresina,
Buenos Aires, Lima, Montevideo, Medellin and Santiago)
in relation to the gold standard. The principal rater from
each centre participated in this stage, and 20 children
aged 7 to 10 years were examined. The kappa ranged
from 0.65 to 1.00 and proportion of agreement from
81% to 100% for the ICDAS II scores. For the other
conditions (PSR, IP and PUFA), training continued until
at least 80% of agreement was reached. The kappa was
not calculated because the sample size did not allow this.

The second step verified the inter-rater reproducibility
between the examiners at each centre in relation to
the principal rater, before examining 20 volunteers per
centre; the kappa value ranged from 0.72 to 0.99 and
proportion of agreement from 96.70% to 98.10% for the
ICDAS II scores. For the PSR, 0.68 to 0.96 and 94.40% to
98.40%, respectively. For the PUFA, the kappa was 1.00
and 100% of proportion of agreement. The values of each
centre are individually represented in Table 1. The repro-
ducibility was measured by the weighted Cohen’s kappa.

At the end of the two calibration steps, a pilot
study was conducted to assess the oral health and an-
thropometry data in four centres: São Paulo, Teresina,

| Centre Test | São Paulo Kappa % of agreement | Teresina Kappa % of agreement | Lima Kappa % of agreement | Santiago Kappa % of agreement |
|-------------|--------------------------------|------------------------------|---------------------------|-------------------------------|
| Inter-rater agreement by centre† | | | | |
| ICDAS II | 0.99199.8 | 0.7298.5 | 0.88496.7 | 0.7998.1 |
| PSR | 0.9699.4 | 0.6898.4 | -² | 0.9594.4 |
| PUFA | 1.0100 | 1.0100 | -² | 1.0100 |
| Intra-rater agreement by centre† | | | | |
| ICDAS II | 0.9599.7 | 0.9299.5 | 0.93499.5 | 0.99199.3 |
| PSR | 0.5375.4 | 0.97698.4 | 1.0100.0 | 0.89994.8 |
| PUFA | 1.0100 | 1.0100 | 1.0100 | 1.0100 |

†The agreement of measure of weight kappa.
²Data not collected.
ICDAS, International Caries Detection and Assessment System; PSR, Periodontal Screening and Recording; PUFA, Pulpal Involvement,
Ulceration, Fistula and Abscess.

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Santiago and Lima. A total of 490 children aged 3 to 10 years (40.8% overweight and 12.9% obese) and 364 adolescents (23.4% overweight and 4.3% obese) aged 11 to 17 years were examined (Tables 2 and 3). The prevalence of dental caries was 76.70% and 81.30%, respectively. Among the children and adolescents with caries lesions, 66.10% and 60.70% had initial lesion caries (criteria 1, 2 and 3) and 34.90% and 25.20% had severe caries lesion, respectively (criteria 4, 5 and 6). In overweight and obese children, the prevalence of dental caries was 72.58% and 65.52%, and adolescents was 46.15% and 50% (Table 3).

The prevalence of gingival bleeding was 49% in children and 58.20% in adolescents; 62.50% of the children and 76.80% of the adolescents showed biofilm on the tooth surface (Table 2). Tables 2 and 3 show the results of the examinations carried out in the pilot study in each centre.

During data collection, 10% of the sample was re-examined for each centre (re-test), and the intra-rater reliability and agreement for ICDAS II ranged between 0.92–0.96 and 98.40–99.50%, while for periodontal conditions PSR 0.53–0.97 and 75.40–98.40% (Table 1)

Table 2 Descriptive data in the pilot study by centre

| Variables            | Children frequency | Adolescents frequency |
|----------------------|--------------------|-----------------------|
|                      | SP n (%)           | TE n (%)              | Li n (%) | SA n (%) |
|                      | n (%)              | n (%)                 | n (%)    | n (%)    |
| School               |                    |                      |          |          |
| Private              | 30 (41.1)          | 89 (44.3)             | 62 (68.9)| 74 (58.7)|
| Public               | 43 (58.9)          | 112 (55.7)            | 28 (31.1)| 52 (41.3)|
| Severe dental caries | 5 (6.9)            | 73 (36.3)             | 45 (50)  | 48 (30.1)|
| ICDAS 4, 5 and 6     |                    |                      |          |          |
| Initial dental caries| 46 (63.0)          | 102 (51.7)            | 88 (97.8)| 88 (69.8)|
| ICDAS 1, 2 and 3     |                    |                      |          |          |
| Gingival bleeding    | 34 (46.6)          | 95 (47.3)             | 35 (38.9)| 76 (60.3)|
| Pocket               | 0 (0)              | 0 (0)                 | 4 (4.4)  | 3 (2.4)  |
| Calculus             | 17 (23.3)          | 8 (4)                 | 5 (6.6)  | 10 (7.9) |
| Pulpal involvement   | 0 (0)              | 0 (0)                 | 0 (0)    | 0 (0)    |
| Presence of biofilm  | 52 (71.2)          | 123 (61.2)            | 81 (92)  | 49 (38.9)|
| Total                | 73 (14.9)          | 201 (41.0)            | 90 (18.4)| 126 (25.7)|

ICDAS, International Caries Detection and Assessment System; Li, Lima; SA, Santiago; SP, São Paulo; TE, Teresina.

Table 3 Distribution of IOTF and oral health conditions in SAYCARE study 2017

| IOTF | Children 3–10 years old | Adolescents 11–17 years old |
|------|-------------------------|-----------------------------|
|      | Normal weight n (%)     | Thinness n (%)              | Overweight n (%) | Obesity n (%) | Normal weight n (%) | Thinness n (%) | Overweight n (%) | Obesity n (%) |
| Caries free | 68 (25.85) | 5 (21.74) | 17 (27.42) | 20 (34.48) | 63 (37.27) | 6 (40.00) | 35 (53.85) | 11 (50.00) |
| Severe dental caries | 145 (50.32) | 12 (44.45) | 38 (51.35) | 25 (39.06) | 61 (36.09) | 4 (26.67) | 28 (43.07) | 7 (31.81) |
| Bleeding | 163 (55.45) | 17 (22.97) | 6 (9.37) | 41 (24.26) | 98 (57.98) | 12 (80.00) | 43 (66.15) | 10 (45.45) |
| Pocket | 30 (10.21) | 4 (14.81) | 18 (24.32) | 10 (15.38) | 7 (4.14) | 1 (6.67) | 5 (7.69) | 1 (4.54) |
| Plaque | 240 (81.63) | 24 (88.89) | 61 (82.43) | 47 (73.43) | 145 (85.79) | 14 (93.34) | 64 (98.46) | 19 (86.36) |
| Total | 294 (64.05) | 27 (5.88) | 74 (16.12) | 64 (13.95) | 169 (62.36) | 15 (5.53) | 65 (23.98) | 22 (8.11) |

Excluding initial and severe lesion caries (ICDAS = 0).

IOTF, International Obesity Task Force; SAYCARE, South American Youth/Child cARdiovascular and Environmental.
Discussion

The inclusion of oral health assessment in the SAYCARE study provided an unprecedented opportunity to study obesity and oral health behaviours of children and adolescents in South America. These children will have their body composition and cardiovascular health monitored, as well as habits that could influence their general health, making it possible to establish causal relationships between obesity and oral health in this population. So it is important to increase the knowledge and verify possible associations, optimizing the collecting data from cultural, economic and lifestyle factors of people from each continent, to know about these diseases and how to prevent them.

Therefore, the proposal of this study was to develop joint methods that allowed evaluation of oral health and obesity measurements, and the present findings have good internal validity, given the inter-rater agreement obtained in the calibration process. The first step in addressing the internal validity and reliability of oral health methods in the present study was to conduct the training and calibration of raters from the six countries involved (São Paulo and Teresina/Brazil, Lima/Peru, Santiago/Chile, Buenos Aires/Argentina, Medellin/Colombia and Montevideo/Uruguay). All raters had previous experience in epidemiological studies, including specific training for ICDA II. The second step was carried out in each centre with the team that would participate in the pilot study and reached good inter-rater agreement that allowed raters to participate in the project data collection. Furthermore, during the pilot study data collection, 10% of the sample was re-examined with the purpose of verifying the intra-rater agreement, which demonstrated the consistency of results collected in different contexts.

The approach and results presented showed the feasibility of developing methods involving the detection of cavitated and non-cavitated carious lesions, periodontal disease and biofilm. In this second stage and in the pilot study, Buenos Aires/Argentina, Montevideo/Uruguay and Medellin/Colombia did not participate due to internal operational difficulties.

There are several statistical approaches that may be used in the measurement of reliability and agreement based on the Guidelines for Reporting Reliability and Agreement Studies (42). In the present study, the weighted Cohen’s kappa was calculated, and the proportion of agreement was used as complementary information (Table 1). Thus, it was assumed that reliability could be defined as the ability of a measurement to differentiate between subjects and objects, and agreement was the degree to which scores or ratings were identical. The weighted kappa calculation provided useful information about reliability of ordinal data such as these in the present study.

Although the sample size was calculated based on the prevalence of diseases, this was a pilot study, and for this reason, no association studies were conducted because their samples could not be considered representative of the population. The main focus of a pilot study should be the feasibility of the larger study rather than the statistical significance (43). Therefore, the methods used to evaluate oral health in the SAYCARE study, the training of examiners and the sample characteristics in the different centres were addressed and discussed.

The methods chosen to assess dental caries and periodontal disease were different of those used in most studies about this theme. Those studies used decayed, missing or filling teeth index for dental caries and periodontal community index for periodontal disease, and the present study used ICDA II and PSR, respectively. This decision was taken considering the sample characteristics, such as age and diseases prevalence in each age group, and also the hypothesis that conflicting results found in literature occurred because the methods did not contemplate the initial stages of caries lesions and did not assess every single tooth regarding the periodontal disease as in the present study. After including initial lesion caries, the evaluation detected twice as many caries lesions, indicating that 65% of overweight and 72% of obese children had at least one caries lesion. In contrast, applying decayed, missing or filling teeth index, 51% and 39% of children would have been diagnosed with dental caries. These oral conditions, identified by using non-invasive tests, may suggest the presence of behavioural indicators for diseases such as obesity and cardiovascular disease that share the same risk factors.

In younger children, the prevalence of periodontal disease was lower; however, when it was present, it was found in its most aggressive form; thus, the method chosen in this study to evaluate it (PSR) allowed detailed tooth-to-tooth examination with three measurements of gingival sulcus on each tooth surface, differently from other methods, such as periodontal community index, widely used in epidemiological studies, which examines only index teeth and not the whole mouth. The more detailed evaluation of periodontal disease in this population, detecting bleeding – the first sign of gingival inflammation – will allow more consistent associations with data related to obesity in a longitudinal study. Gingival bleeding is related to both periodontal disease and dental caries because it is caused by the presence of dental biofilm and indicates poor oral hygiene. In the present study, adolescents presented higher prevalence of gingival bleeding (58.20%) than children (49%), indicating that...
inflammation progresses with age (44). In addition, the prevalence of calculus (23.60%) in adolescents was also higher than in children (8.20%).

The limitations of this study to be corrected in future studies were due to the PUFA and biofilm quantity indicators. PUFA was trained in the first calibration stage; however, there were not many cases in the second stage, which led to a 100% agreement in the existing cases. Therefore, it will be necessary to pre-select the cases for the training period, prior to the study. Relative to presence of biofilm, a separate group of volunteers will be necessary for this training because the ICDAS examination required previous prophylaxis; thus, only the first examiner was able to measure this variable.

The presence of both dental biofilm and sucrose in the diet is mandatory conditions for the development of dental caries. There is a general agreement that dental caries should be controlled from its earliest stages, as the history of the disease is considered a risk predictor for the development of future lesions. Diet has an influence on both oral health and obesity (13), and the correlation between them has been widely studied; however, the results are conflicting. Nevertheless, the majority of both cross-sectional and longitudinal studies did not consider early stages of caries lesions as in the present study. The hypothesis for the longitudinal study is that by evaluating the initial stages of dental caries, periodontal disease and obesity, and controlling confounding effects, it would be able to advance in this investigation.

Conclusion
The results of the present study demonstrated good reproducibility and internal validity after the training of the examiners, as well as the feasibility of using the methods chosen for this multicentre study.

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Conflict of interest statement
The authors declared no conflict of interest.

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