ACTA ODONTOLOGICA SCANDINAVICA
2018, VOL. 76, NO. 7, 509–514
https://doi.org/10.1080/00016357.2018.1444201

Taylor & Francis
Taylor & Francis Group

ORIGINAL ARTICLE

Relationship between depth of approximal caries lesions and presence of bacteria in the dentine in primary and permanent posterior teeth: a radiographic examination with microbiological evaluation

Azam Bakhshandeh[a], Isabela Floriano[b], Mariana Minatel Braga[b], Kristine Arnholdt Thorlacius[c] and Kim Rud Ekstrand[a]

[a]Section of Cariology and Endodontics, Department of Odontology, University of Copenhagen, Copenhagen, Denmark; [b]Department of Paediatric Dentistry, School of Dentistry, University of Sao Paulo, Sao Paulo, Brazil; [c]Public Dental Health Care Service, Municipality of Høje-Taastrup, Høje-Taastrup, Denmark

ABSTRACT

Objectives: We aimed to determine the relation between the radiographical depth of approximal lesions and the presence of bacteria in the dentine in posterior teeth in both dentitions.

Material and methods: Sample 1 consisted of 34 approximal lesions in primary molars in children aged 5–7 years old. Sample 2 consisted of 48 approximal lesions in molars and premolars in adult patients aged 18–67 years old. All lesions were in need of restorative treatment according to the dentists. During the operative interventions dentine biopsies were collected with a sterile bur just pulpally of the enamel–dentin junction. Two authors evaluated the presence/absence of bacterial colonies. The lesions depth on bitewing radiographs ($R_{SCORING}$) were assessed independently by two examiners twice using the ICCMS classification system: R_I=initial-; R_M=moderate-; R_E=extensive caries.

Results: In sample 1, the $R_{SCORING}$ was distributed as follows: R_I=15; R_M=12; RE = 7. In 9 cases the lesions were clinically cavitated. Bacteria were visible on the agar plates in one case (7%) of the R_I lesion, 86% of the R_M lesions and in all the R_E lesions, ($p < .001$). In sample 2, $R_{SCORING}$ was distributed as follows R_I=14; R_M=23; R_E=9. In 15 cases, the lesions were clinically cavitated. In 2 cases (14%), there were visible bacteria on the agar plates among the R_I lesions, while this was the case in 86% of R_M lesions and in 100% of R_E lesions ($p < .001$).

Conclusions: R_M and R_E lesions seem to harbor bacteria in the dentine and are candidates for invasive treatment. In contrast, R_I lesions should in general be managed non-invasively.

ARTICLE HISTORY
Received 8 December 2017
Revised 13 February 2018
Accepted 18 February 2018

KEYWORDS
Approximal caries; bacteria; bitewing; clinical assessment; caries treatment

Introduction

A recent summary showed a large variation between dentists around the world with regards to assessing the threshold for invasive treatment on approximal caries lesions [1]. Thus, about 50% of the dental responders would intervene invasively when the approximal lesion on radiographs was confined to the enamel, while the other 50% would wait until the lesion had crossed the enamel dentine junction and thus extended into the dentine. This large variation and extreme tendency to initiate invasive treatment on such early lesions call for new detection criteria to avoid overtreatment of approximal lesions. It is very difficult to detect early breakdowns in approximal enamel *in vivo* [2,3], if separation is not used. Therefore, it could be speculated that knowledge regarding at which radiographic lesion depth approximal lesions in both primary and permanent molar teeth harbour bacteria in the dentine, could be beneficial for clinicians when deciding when to intervene invasively or non-invasively. This study hypothesizes that bacteria are present in a significant amount in the dentine, but first after the radiolucency at the approximal lesion has extended the outer one-third of the dentine.

The aim of this study was to determine the relation between the radiographic depth of the approximal lesion and the presence of bacteria in the dentine in molar teeth in the primary dentition and in molar and premolar teeth in the permanent dentition. Lesion depths on the radiographs were classified by the ICCMS radiographic classification system [4,5].

Material and methods

The study was carried out at the Section of Cariology and Endodontics, University of Copenhagen, Denmark (UCPH) from 2015 to 2017. Two samples were included: sample 1 consisting of primary teeth and sample 2 of permanent teeth.

Approval to perform the study was granted from the Danish Data Protection Agency (J-nr.: Sund-2017-04). A signed consent form from the parents to the children in

CONTACT Azam Bakhshandeh azamba@sund.ku.dk Section of Cariology & Endodontics, Department of Odontology, University of Copenhagen, Nørre Allé 20 DK, 2200 Copenhagen N, Denmark

sample 1 was required before the children could be included in the study. Similarly, signed consent forms were used for the adults in sample 2.

Sample size considerations

In a previous paper [6] where the examination was conducted on occlusal surfaces, data showed that 70% of lesions with a radiolucency depth limited to the outer one-third of the dentine (initial lesions) harboured bacteria at the enamel–dentine junction. The amount of bacteria was, however, very small and significantly less than lesions with the radiolucency deeper than the outer one-third of the dentine (moderate or extensive lesions). Thus, for the actual study, we estimated that bacteria are present just pulpal to the enamel–dentine junction in an amount that they are visible on agar plates in less than 25% of the initial lesions and in more than 75% of the moderate to extensive lesions. With a chosen β on 80%, $\alpha = 5\%$, and using the formula for comparing two proportions in independent groups and Lehr's formula [7], it was estimated that about 15 initial lesions and 15 moderate or extensive lesions should be included in the study.

Inclusion criteria

Sample 1 included children aged 5–7 years old with the following characteristics: they had a lesion that without any doubt was limited to the approximal surface on a primary molar tooth assessed on radiograph; the lesion, required invasive treatment according to the dentist, who was responsible for the dental care of the child and based on the clinical and radiographical information (individual dentists' decision-making process). In sample 2, the participants were patients who voluntarily came for treatment at the dental school in Copenhagen. Inclusion criteria: 18 years or older and with the following characteristics: they had a lesion, which radiographically and without doubt was solely developed from the approximal surface on a molar or a pre- molar tooth; the lesion required invasive treatment according to the dentist, who was responsible for the dental care of the participant and based on the clinical and radiographical information (individual dentists' decision-making process).

In both samples, if the participant had more than one approximal lesion requiring invasive treatment on the included tooth, the surface to be included in the study was randomly determined (number table) and the other/others was/were treated, but not included in the data for this study. Participants with chronic diseases or handicaps were not included in the study.

Settings

Cooperation between the Section of Cariology and Endodontics and the Public Dental Health Service (PDHS) in Copenhagen, Denmark was established in order to allocate children for sample 1. Three dentists from the same clinic close to the dental school performed all the treatments. Four dentists from the Section of Cariology and Endodontics handled the treatment of the selected patients in sample 2.

Radiographs were available at the time of the clinical investigation. Digital radiographs using phosphor plates (Digora, Soredex, Finland) were taken by skilled and experienced dental assistants. The exposure time for children was 0.126 sec. (60 kV, 7 mA) and for adults was 0.145 sec. (70 kV, 8 mA). All radiographs were scanned (Digora^{TM} Optime, Soredex, Finland) and stored electronically in the patient file.

According to the Danish National Board of Health, each observed carious lesion requires a diagnosis. In the PDHS, the lesion is described clinically as cavitated or not-cavitated [8]. Furthermore, if radiographs are available, the lesion can be defined as present in the enamel only or extending into the dentine. A final diagnosis is established with regards to whether the lesion is initial or manifest, the latter meaning a cavitated or not-cavitated lesion, but radiographically well into the dentine. At the Section of Cariology and Endodontics, the lesion is clinically divided into three stages: (a) not-cavitated, (b) enamel breakdown/shadowed or (c) dentine cavitated. Furthermore, the lesions were classified as active or inactive using Ekstrand criteria that determine that presence of gingival bleeding is a reliable indicator for lesion activity [8]. If radiographs were available, the lesion (radiolucency) was defined as: in enamel only, in the outer one-third of the dentine, in the middle one-third of the dentine or in the inner one-third of the dentine. A final diagnosis is established in terms of initial, moderate or extensive [9,10].

Sampling of dentine on teeth in need of invasive treatment [modified from 11]

All involved dentists received a theoretical instruction (PowerPoint) in collecting the dentine sample. Photographs showed how to access the dentine, control for saliva/blood contamination by means of approximal protectors (FenderWedge, Directa AB, Sweden), how to place the bur, how to fill the bur with dentine tissues (no water/air) and finally how to transfer the bur to the transport media. The dentists were supervised clinically by one author (KRE) during one or two cases.

In cases when the selected lesion did not have a clinically detected cavitation, access to the enamel dentine junction (EDJ) was achieved through the marginal ridge by a high-speed air rotor with a diamond bur. Samples of dentine were collected just pulpally to the EDJ in one corner of the class I–II boxes with a sterile, stainless steel bur (1 mm in diameter) at low rotation in a hand piece. In already cavitated lesions overlying carious enamel and dentine were removed with a low speed bur and the sample (with a sterile bur) was collected immediately at the EDJ. After sample collection, the bur was carefully removed from the hand piece and placed into a container with 5 ml phosphate-buffered saline water (pH = 7) and taken to the laboratory for processing.

All samples were processed in the laboratory within 1 h after collection.

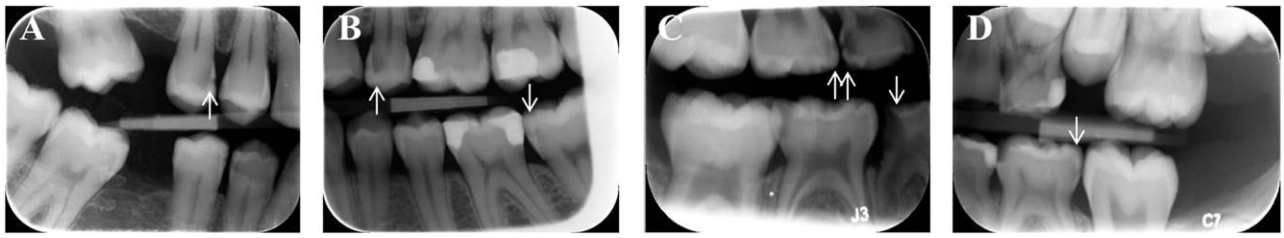

Figure 1. Radiographic classification of the caries lesions in permanent and primary teeth.

Radiographic classification of lesions

Two authors (IF, KRE) independently assessed the lesion depth radiographically using the 4-point International Caries Classification and Management System (ICCMS™), [9–12]. Assessments were performed on a computer screen (Olôrin, Sweden) in a diagnostic room with dark conditions. As no sound surfaces were included in this study, only the following three scores were used (Figure 1):

R_I = initial (radiolucency in enamel and if in dentine up to one-third of coronal dentin towards the pulp)
R_M = moderate (radiolucency in one-third to two-third of coronal dentine towards the pulp)
R_E = extensive (radiolucency more than two-third of coronal dentine towards the pulp)

Radiographic classification of the lesions ($R_{SCORING}$) was performed twice by two authors (IF and KRE), with a least 1 week in between.

Microbial procedure

The basic agar medium employed for identifying microorganisms was Todd-Hewitt broth (Difco Lab., Detroit, MI) supplemented with 17g/L Bacto agar (Difco Lab., Detroit, MI) and 20 mM glucose. Then, autoclaving and cooling to 45°C was carried out, sterile 1 M citratephosphate buffer was added to a final concentration of 20 mM, and the pH adjusted dropwise with sterile 5*N* HCl or 8*N* KOH for eventually creating agar plates adjusted to a pH of 7.0, 6.5, 5.5 or 4.5. These 'pH agar' plates were used within two days and the pH of the agar was tested with a pH surface electrode just prior to use.

The container with the bur was shaken by hand for 10 s in order to dislodge the adherent dentine sample from the bur. Samples were vortex-mixed within 1 h of collection, and 100 µl of the suspension (undiluted) was inoculated onto each of the pH agar plates ($n = 4$). The plates were incubated anaerobically for 7 days. Images were taken of the plates after the seventh day.

Two examiners (AB, KRE), blinded for tooth number and their radiographs, evaluated the images of the agar plates ($n = 4$ for each sample) with regards to the presence or absence of bacterial colonies. The evaluation was performed twice, with a least one-week interval.

Statistical analysis

The data were analysed using statistical software (MedCalc 15.11, MedCalc Software, Belgium). From the patient files, data concerning the clinical stage of the selected lesions were collected with regards to whether the lesions were cavitated or not-cavitated. The registration of cavity versus no cavity was registered by the dentists at the clinics.

Intra- and inter-examiner agreements (first assessments) of $R_{SCORINGS}$ were assessed, using weighted Kappa.

Analyses of all the agar plates (undiluted; $n = 320$) showed that if bacteria were observed on one plate from a participant, bacteria would also be observed on the three other plates. The same was the case when no bacteria were observed. Therefore, it was decided to use the plates adjusted to pH 4.5 in order to assess the presence or absence of bacteria for this study. Intra- and inter-examiner agreement of the presence and absence of bacteria were conducted on the adjusted pH 4.5 plates using percentage of perfect agreement. The relationship between $R_{SCORINGS}$ and the presence of bacteria was assessed using Fisher's exact test. Similar calculations were carried out concerning clinical status of the lesion and presence or absence of bacteria. For these analyses, the statistically significant level was set at 0.05.

Results

General description

Sample 1: A total of 34 approximal lesions on primary molar teeth were operatively treated. Two-thirds of the sample came from girls. The 34 molar teeth were equally distributed between upper and lower molar teeth. In 23 cases (68%) the sample was collected from distal surfaces and in 11 cases (32%) from the mesial surfaces. The $R_{SCORING}$ from KRE's first assessment was distributed as follows: R_I=15 (44%); R_M=12 (35%); R_E=7 (21%), (Table 1). Internal analyses disclosed that all 15 of the R_I lesions had progressed into the dentine and that all seven teeth scored R_E, clinically appeared cavitated. This was also the case for two of the R_M lesions, which means that nine of the 34 lesions (36%) were clinically cavitated. The presence of bacteria on the adjusted 4.5 pH agar plates was observed in 17 (50%) of the samples.

Sample 2: A total of 48 lesions were operatively treated. Fifty-nine per cent of the sample came from males. Two-thirds of the lesions were from the upper jaw. Fifty-three per cent were molar teeth and 47% premolar teeth. The $R_{SCORING}$ from KRE's first assessment was distributed as follows: R_I=14; R_M=24; R_E=10 (Table 1). Internal analyses disclosed that all the 14 of the R_I lesions had progressed into the dentine; and that all 10 teeth scored R_E clinically appeared cavitated. This was also the case for 5 of the R_M lesion. Thus, a total of 15

Table 1. Lesion depth (R_I, R_M, R_E) in absolut numbers and frequencies of cases where presence of bacteria were noted related to the different lesion depth in both samples.

	R_I (*n*) / C(*n*)/ P(*n*)	R_M (*n*) /C(*n*)/ P (*n*)	R_E(*n*) /C(*n*)/P (*n*)
Sample 1 (*n* = 34)	15 / 0 / 1	12 / 2* / 7	7 / 7* / 7
Sample 2 (*n* = 48)	14 / 0 / 2	24 / 5* / 21	10 / 10* / 10

*Note that all cavitated lesions showed visible bacteria on the agar plates.
C: Clinical assessment – Cavitated lesion; P: Microbiological assessment – Presence of bacteria; R_I: Radiographical assessment – Initial lesion; R_M: Radiographical assessment – Moderate lesion; R_E: Radiographical assessment – Extensive lesion.

of the 48 (31%) lesions were clinically cavitated. The presence of bacteria on the agar plates was observed in 31 (67%) of the samples and in all cavitated lesions.

Reproducibility of the measurements and assessments of lesions

The intra-examiner reproducibility for the $R_{SCORINGS}$ was 0.88 for IF and 0.89 for samples 1 and 2, respectively. Similar figures for KRE were 0.93 and 0.88. Inter-examiner agreements using the first set of readings for both samples were 0.88.

The intra-examiner percentages of agreement in assessing the plates (the presence or absence of bacteria) for both examiners were ≥98%. The inter-examiner agreement using first sets of readings was 98% for sample 1 and 99% for sample 2.

Relationship between lesion depth and presence of bacteria in dentine

Table 1 presents the $R_{SCORINGS}$, divided into R_I-, R_M-, or R_E-lesions for both samples. For each radiographical scoring, the number of lesions clinically assessed to be cavitated (C) was noted. Furthermore, the presence of bacteria on the agar plates (P) was registered as well.

None of the R_I lesions were cavitated and in only 1 case from sample 1 (7%) and 2 cases (14%) from sample 2 bacteria were present on the agar plates. In the primary teeth, only two (17%) of the R_M lesions were clinically cavitated, but the presence of bacteria on the agar plates was noted in seven cases (58%). Similar data from the permanent dentition showed that five (21%) of the R_M-lesions were cavitated, but the presence of bacterial on the agar plates were noted in 21 cases (88%). All R_E lesions (in both dentitions) were cavitated and the presence of bacteria was observed on all agar plates. Fisher exact tests revealed that the presence of bacteria in the dentine was significantly associated with $R_{SCORINGS}$ where the lesions were deeper than the outer one-third of the dentine (R_M or R_E lesions, Table 1). This included both primary ($p = .0001$) and permanent teeth ($p = .000001$). Further analyses disclosed that the presence of bacteria was also associated with R_M lesions and not with R_I lesions both in the primary ($p = .008$) and in the permanent dentition ($p = .000001$). No differences were seen with respect to R_M and R_E in this context (Primary teeth, $p = .11$ and permanent teeth, $p = .5$). Finally, the presence of bacteria on the agar plates was significantly associated with cavitated lesions (Primary teeth, $p = .002$ and permanent teeth, $p = .0001$).

Discussion

In the recent review referred to in the introduction [1], very few Scandinavian dentists would intervene invasively on an approximal lesion confined to the enamel or even when the lesion was at the EDJ. In general, Danish dentists perform invasive treatment when a lesion is clinically cavitated, thus well into the dentine [13] or when a lesion radiographically is well into the dentine. In the present study, all lesions included were radiographically into the dentine.

When combining observations from both samples, about one-fifth of the sample included in this study was characterized by the dentists as being clinically cavitated lesions. Bacteria were observed on all the related agar plates, which is not surprising [14,15]. About 80% of the included lesions were clinically non-cavitated due to the dentists, but all had dentine caries radiographically. In only three cases (10%), visible bacteria were noted on the agar plates in the radiographically characterized initial lesions (R_I), while this was the case in 78% of the radiographically moderate-staged lesions (R_M). If the presence of bacteria in the dentine (detected on the agar plates) is used as a threshold for performing operative dentistry, then excessive treatment is the result in about 20% of the radiographically moderate-staged lesions. In contrast, insufficient treatment is the result in 10% of the initial-staged lesions. This should be seen in relation to the fact that if only the clinically cavitated lesions were treated operatively, insufficient treatment would have been performed in 21 out of the 28 (75%) moderate-staged lesions (clinically not cavitated), where visible bacteria could be detected on the agar plates. The question is whether a more thoroughly clinical investigation could lead to the detection of more "cavitated" approximal lesions. It has to be emphasized that caries in Denmark, where this study was conducted, is characterized as a slowly progressing disease. One of the reasons for this is that almost all Danes, including children, brush their teeth with fluoridated toothpaste 1 to 2 times per day [13]. Thus, the development of breakdowns in the surfaces enamel or even cavitated dentine lesions is postponed under slowly progressing conditions compared to locations where the progression rate is faster [16,17]. The results from this study imply that bitewing radiographs provide a more precise picture of the depth of the approximal lesion than traditional visual examination (not using separation). Furthermore, radiographs are useful to decide if bacteria are present in dentin at least at level we found on the agar plates.

Study validation

Three dentists from the Pediatric Dental Health Care System and four dentists from the section of Cariology and

Endodontics took part in this study. The dentists working in the Pediatric Dental Health Care System are all clinically calibrated in scoring between non- and cavitated caries lesions and radiographically calibrated to assess whether lesions are in the enamel or dentine. With regards to treatment decisions, dentists are allowed to decide when to restore a lesion. The dentists who performed the treatment on the adults (sample 2) are calibrated in the ICCMS R_{SCORE} system [9,10], and clinically in differentiating between non-cavitated lesions, enamel cavitation or shadowed lesions and dentine-cavitated lesions. The dentists involved in this study treat patients 3–4 times per week and are experienced in diagnosing caries radiographically and clinically and to manage caries on the patients.

All the participating dentists achieved good experience in the process of taking the dentine biopsy. The authors supervised them on one or two occasions. The dentine biopsy taken by a sterile bur is a well described method [18]. The fact that so many initial lesions were non-infected (90%) at a level so we could notice it on the related agar plates and the fact that 78% of the media lesions and all (100%) extensive lesions were infected suggested that the dentists could handle the process well without e.g. artificially contaminating the bur.

The radiographs were all taken by experienced professionals. In only a few cases, the radiographs were not orthoradial on the tooth. High intra- and inter-reproducibility values were achieved (Kappa $\geq$88) when classifying the radiographs into the three scores (R_I, R_M and R_E –lesions). KRE showed the highest intra-reproducibility, which is why the first of his readings were used as variable for both sample 1 and sample 2.

The intra- and inter-reproducibility of reading the agar plates were nearly 100% for both examiners.

In the present study, we used the same microbiological set-up as Svensäter et al. [19] with the exception that the present study only operates with four levels of pH at the agar plates (7.0, 6.5, 5.5 or 4.5). Our aim was not to verify special cariogenic species in the dentine, but to make sure that the microorganisms from the samples were able to survive in aciduric conditions. In all cases, bacteria were seen on the agar plates, thus indicating that the dentine was infected. Bacteria were logically found at all pH levels, in accordance with the definition that the cariogenic plaque is an 'overgrowth of acid-producing and acid-resistant members of the normal oral flora' as stated by Shellis [20]. This justified why we decided to use only agar plates adjusted to pH 4.5 in order to relate to the depth of the lesions in this study.

To test the validity of the microbiological method, we included four primary first molar teeth, freshly extracted due to caries. However, the mesial surfaces had no radiographical caries due to the narrow contact to the canines. The microbiological examination disclosed that bacteria were not found on the agar plates in any of these 'lesions'.

Lack of bacteria on the agar plates does not indicate, though, that no bacteria are present in the enamel or at the enamel dentine boarder in the initial characterized lesions. Other studies have shown that even in enamel lesions bacteria can be present in the enamel [21,22]. What we can assume is that there are no or so few bacteria within the dental tissues that it might not influence the progression rate under the condition that action is taken to control the biofilm on the surface.

In conclusion, a significant correlation was found between radiographic lesion depth and presence of bacteria in the dentine. Lesions corresponding to R_M or R_E in the ICCMS^{TM} system seem nearly always to harbour bacteria in number sufficient to be visible at the agar plates. These lesions are likely to be treated with invasive approaches. When the lesion is classified as an initial lesion (R_I) with the ICCMS^{TM} harbouring demineralized enamel or also demineralization of the outer one-third of dentine, but with no visible bacteria on the agar plates, the lesion can and should be non-or micro-invasively managed.

Acknowledgements

The authors express their gratitude to Professor Ivar Espelid, Faculty of Dentistry, Institute of Clinical Dentistry, Norway, for his valuable advice for improving this paper. Associate Professor Tove Larsen, Department of Odontology, Faculty of Health and Medical Sciences, University of Copenhagen, Denmark is acknowledged for advice concerning microbiology. Technical assistant Mia Linding Ekstrand is thanked for her excellent work with the microbiological evaluation. None of the authors has any financial or personal conflict of interest to declare.

Disclosure statement

The authors declare no conflict of interest.

The study was conducted during Isabela Floriano's split fellowship program at the University of Copenhagen, supported by the National Council for Technological and Scientific Development (CNPq Science without Borders 203244/2015-0). Funding parties had no role in the study design, data collection and analysis, decision to publish or preparation of the manuscript.

Funding

This work was supported by the Research of Sao Paulo (FAPESP 2013/27206-8) and a grant from Colgate Palmolive.

ORCID

Isabela Floriano http://orcid.org/0000-0002-7352-5655

References

[1] Innes NPT, Schwendicke F. Restorative thresholds for carious lesions: systematic review and meta-analysis. J Dent Res. 2017; 96:501–508.

[2] Pitts NB, Rimmer PA. An *in vivo* comparison of radiographic and directly assessed clinical caries status of posterior approximal surfaces in primary and permanent teeth. Caries Res. 1992;26: 146–152.

[3] Hintze H, Wenzel A, Danielsen B, et al. Reliability of visual examination, fibre-optic transillumination, and bite-wing radiography, and reproducibility of direct visual examination following tooth separation for the identification of cavitated carious lesions in contacting approximal surfaces. Caries Res. 1998;32:204–209.

[4] Pitts NB, Ekstrand KR. ICDAS Foundation. International Caries Detection and Assessment System (ICDAS) and its International Caries Classification and Management System (ICCMS) – methods

for staging of the caries process and enabling dentists to manage caries. Community Dent Oral Epidemiol. 2013;41:e41–e52.
[5] Pitts NB, Zero DT, Marsh PD, et al. Dental caries. Nat Rev Dis Primers. 2017;25:3.
[6] Ricketts DN, Ekstrand KR, Kidd EA, et al. Relating visual and radiographic ranked scoring systems for occlusal caries detection to histological and microbiological evidence. Oper Dent. 2002;27: 231–237.
[7] Petrie A, Sabin C. Medical statistics at a glance. 3rd ed. Oxford: Wiley Blackwell; 2009.
[8] Nørrisgaard PE, Qvist V, Ekstrand KR. Prevalence, risk surfaces and inter-municipality variations in caries experience in Danish children and adolescents in 2012. Acta Odontol Scand. 2016; 74:291–297.
[9] Ismail AI, Pitts NB, Tellez M, Authors of International Caries Classification and Management System (ICCMS). The International Caries Classification and Management System (ICCMS^{TM}) an example of a caries management pathway. BMC Oral Health 2015;15(Suppl 1):S9.
[10] Pretty IA, Ekstrand KR. Detection and monitoring of early caries lesions: a review. Eur Arch Paediatr Dent. 2016;17:13–25.
[11] Kühnisch J, Ekstrand KR, Pretty I, et al. Best clinical practice guidance for management of early caries lesions in children and young adults: an EAPD policy document. Eur Arch Paediatr Dent. 2016;17:3–12.
[12] Cortes A, Ekstrand KR, Gamboa LF, et al. Caries status in young Colombian children expressed by the ICCMS^{TM} visual/radiographic combined caries staging system. Acta Odontol Scand. 2017; 75:12–20.
[13] Ekstrand KR, Bruun G, Bruun M. Plaque and gingival status as indicators for caries progression on approximal surfaces. Caries Res. 1998;32:41–45.
[14] Qvist V. Resin restorations: leakage, bacteria, pulp. Dent Traumatol. 1993;9:127–152.
[15] Ratledje DK, Kidd EAM, Beighton D. A clinical and microbiological study of approximal carious lesions. Caries Res. 2001;35:3–7.
[16] Sansare K, Raghav M, Sontakke S, et al. Clinical cavitation and radiographic lesion depth in proximal surfaces in an Indian population. Acta Odontol Scand. 2014;72:1084–1088.
[17] Wenzel A. Radiographic display of carious lesions and cavitation in approximal surfaces: advantages and drawbacks of conventional and advanced modalities. Acta Odontol Scand. 2014;72: 251–264.
[18] Kidd EA, Joyston-Bechal S, Beighton D. Microbiological validation of assessments of caries activity during cavity preparation. Caries Res. 1993;27:402–408.
[19] Svensäter G, Borgströmb M, Bowdenc GHW, et al. The acid-tolerant microbiota associated with plaque from initial caries and healthy tooth surfaces. Caries Res. 2003;37:395–403.
[20] Shellis P. Etiology and patogenesis of caries. In: Meyer-Lueckel H, Paris S, Ekstrand KR, editors. Caries management - science and clinical practice. New York: Thieme; 2013. p. 21–38.
[21] Mejàre I, Brännström M. Deep bacterial penetration of early proximal caries lesions in young human premolars. ASDC J Dent Child. 1985;52:103–107.
[22] Parolo CC, Maltz M. Microbial contamination of noncavitated caries lesions: a scanning electron microscopic study. Caries Res. 2006;40:536–541.