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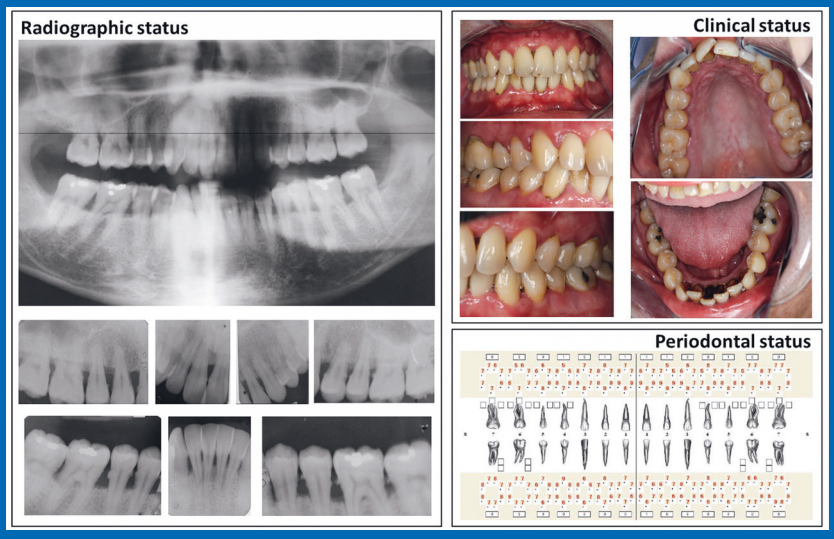

Using clinical cases to stimulate active learning in a short periodontal continuing professional development course **page 1**

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Using clinical cases to stimulate active learning in a short periodontal continuing professional development course

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Abstract

◎ A case-based approach was used in a two-day periodontal continuing professional development course as a strategy to stimulate active learning. The present study investigates the outcome of this course format in terms of feasibility, perceived efficiency as a learning approach and reported individual learning goals.

The study was performed in five identical courses entitled 'risk analysis and treatment in periodontal patients' at Malmö University between 2011-2014. Before the course, clinical cases were used to activate participants' prior knowledge and to attune their focus on the course content. During the course, cases were discussed to synchronise theory with practical application. A pre- and end-course questionnaire were developed to evaluate participants' characteristics (age, clinical expertise, experience and expectations), perceptions on feasibility and instructiveness and emerged individual learning goals.

The participants (39 dentists and 78 dental hygienists) reported an average preparation time of 62 minutes (range 2-190) and had positive perceptions on the accessibility, instructiveness and difficulty of cases. Expectations ranged between refreshing, acquiring new knowledge and mastering the course subject. Most reported learning goals were related to daily clinical practice including the development of a treatment plan, when to continue non-surgical treatment or to extract teeth/perform surgery, the approach to periodontitis, how to motivate non-compliant patients and when to refer.

Conclusion: The use of clinical cases to stimulate active learning in a short-term continuing professional development periodontal course was positively perceived by the dentists and dental hygienists in terms of feasibility and learning potential.

Key words

Case-based learning, continuing professional development, periodontology, dentists, dental hygienists

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Användning av kliniska fallbeskrivningar för att stimulera aktiv inlärning vid korta professionella efterutbildningskurser inom parodontologi

Sebastiaan Koole, Eric Thevissen, Ulf Lindén, Björn Klinge, Hugo de Bruyn

Sammanfattning

◎ I denna studie användes fallbeskrivningar vid korta professionella efterutbildningskurser inom parodontologi, som en strategi för att stimulera aktiv inlärning. I undersökningen utvärderas och redovisas utfallet av denna undervisningsform avseende genomförbarhet, upplevd effektivitet som inlärningsmetod och rapporterade individuella inlärningsmål.

Studien genomfördes i anslutning till efterutbildningskurser som handlar om riskbedömning och behandling av parodontit. Kurserna genomfördes vid Odontologiska fakulteten, Malmö högskola mellan 2011-2014. Före kursstart fick deltagarna tillgång till kliniska fallbeskrivningar för att aktivera tidigare kunskaper och för att inrikta deras fokus på kursens innehåll. Under kursen presenterades och diskuterades kliniska fall för att koppla teori med praktisk tillämpning. Ett frågeformulär utarbetades och användes före och efter kursen. På detta sätt utvärderades bakgrundsvariabler (ålder, profession, kön, klinisk erfarenhet etc.), förväntningar och uppfattning om genomförbarhet och relevans och uppkomna individuella inlärningsmål.

Deltagarna (39 tandläkare – 78 tandhygienister) redovisade en genomsnittlig förberedelsetid på 62 minuter (spridning 2-190 minuter) och hade en positiv uppfattning om tillgänglighet, tydlighet och fallens svårighetsgrad. Förväntningarna sträckte sig från aktualisering av tidigare kunskaper, att förvärva nya kunskaper till att bemästra kursinnehållet. Merparten av angivna inlärningsmål var kopplade till daglig klinisk verksamhet, som innefattar att redovisa en behandlingsplan, att bedöma när icke-kirurgisk mekanisk instrumentering skall fortsätta, eller när tänder skall extraheras eller kirurgi skall genomföras, hantering av parodontitpatienten, motivera icke-samarbetande patienter eller när remiss är aktuellt.

Slutsats: Användning av kliniska fallbeskrivningar för att stimulera aktiv inlärning vid korta professionella efterutbildningskurser inom parodontologi upplevdes positivt av deltagande tandläkare och tandhygienister vad avser genomförbarhet och inlärningsmöjligheter.

Introduction

To ensure high quality standards of care, dentists and dental hygienists need to engage in a lifelong learning trajectory, continuously optimizing own competence and to be informed about the most recent developments in the clinical field (2, 14). In response, many educational institutions organize short term continuing professional development courses to provide these professionals with updates about the newest insights on periodontal diseases, methods to diagnose and/or treatment options. Often these courses are lecture-based, in which participants passively listen to experts presenting the information (9).

This rather traditional approach to learning has been identified as inefficient and incongruent with the present perspective on learning which advocates for a dynamic learning strategy (18). This inquires participants to get actively involved in processing the learning content, which is reported to result in deeper learning and consequently an increased knowledge uptake as well as long-term retention of the received information (7, 13, 19). Translated into the context of a periodontal continuing professional development course, this suggests that active consideration of prior knowledge and learning goals in advance and discussing learning content in relation to practical implications during a course will create a goal-orientated focus. This will lead to an enhanced assimilation of the given topics in the course.

In clinical education, cases are often used to illustrate the application of theory or practice in a professional environment. Discussing clinical situations without the pressure of a patient expecting an immediate response to a question or given problem has the advantage to create a safe environment. It enables a profound analysis of patient-related factors as well as scrutiny of treatment options including alternatives (1, 20). Consequently case-based learning is considered a valuable approach to foster clinical reasoning and reflection (8).

In attempt to create a dynamic learning environment in a periodontal continuing professional development course entitled 'Risk analysis and treatment in periodontal patients' at Malmö University, illustrated written clinical cases were introduced to stimulate active learning. Clinical cases were sent to the participants in advance of the course to activate their prior knowledge and to attune their focus on the learning content. During the course, the provided cases were used to trigger clinical thinking and to synchronize theory and practice.

To investigate the outcome of this course format in terms of feasibility, perceived efficiency and reported individual learning goals of the participating dentists and dental hygienists, five research questions were proposed.

1. What is the feasibility of introducing a case-based format in advance of a short-term continuing professional development periodontal course, concerning spent preparation time and the delivery of clinical cases?
2. How do dentists and dental hygienists perceive the efficiency of a case-based format in a short-term continuing professional development course?
3. Do dentists and dental hygienists perceive the efficiency of a case-based format differently in relation to their clinical expertise, years of clinical expertise as well as spent pre-course preparation time?
4. Do perceptions about cases as a preparation strategy to follow this treatment-planning course change during the lecture?
5. What individual expectations and learning goals do dentists and dental hygienists propose in advance of a short-term continuing professional development course?

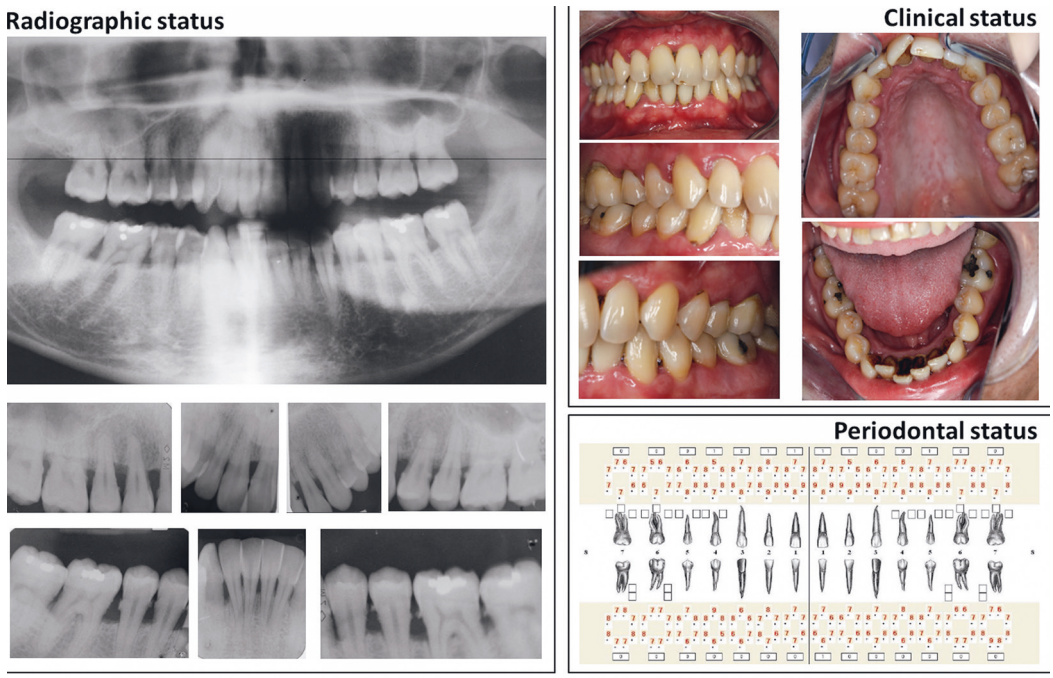
Material and Methods

At Malmö University, Faculty of Odontology each year a two-day (12 hours) continuing professional development course in periodontology is organized. This course, entitled "Risk analysis and treatment in periodontal patients", is intended for dentists and dental hygienists. A case-based educational strategy is used to stimulate the participants to engage in active learning. Before the onset of the course, participants received five clinical cases via the internet with a question to formulate a treatment plan and prognosis. These cases had the purpose to activate participants' prior knowledge and to attune their mind-sets to the course content. During the course, these cases were extensively discussed to synchronise theory with practical application. Each case was based on a real treated patient and provided clinical information including a full-mouth peri-apical radiographic status, an orthopantomogram, clinical status and a full-mouth periodontal chart (10). The latter provided information on the presence of plaque, bleeding, furcation involvements and tooth mobility. Furthermore it allowed the determination of a prognosis of all individual teeth. Besides clinical information a brief description of the patient's risk factors and main complaints and/or demands was

◎ **Table 1.** Overview of case characteristics, including the clinical information in the case and diagnosis and treatment actual performed in the patient.

	Case I	Case II	Case III	Case IV	Case V
CLINICAL INFORMATION					
Age	15	65	45	32	32
Plaque index	100 %	81 %	86 %	20 %	100 %
Bleeding index	100 %	20 %	61 %	65 %	100 %
No. teeth (wisdom teeth excl.)	28	26	19	32	28
Deepest pocket	5 mm	8 mm	9 mm	9 mm	9 mm
Maximum recession	0 mm	4 mm	3 mm	0 mm	3 mm
Maximum mobility	None	Degree II	None	Degree III	Degree I
Furcation involvement	None	Degree III	Degree III	Degree III	Degree I
Risk factors	None	None	Light smoking	History depression	Heavy smoking
DIAGNOSIS	Plaque related gingivitis	Chronic periodontitis	Chronic periodontitis	Generalised aggressive periodontitis	Localised aggressive periodontitis
TREATMENT					
Oral hygiene instruction	X	X	X	X	X
Scaling and Rootplaning	X	X	X	X	X
Extraction		5 teeth	2 root resections	5 teeth	4 teeth
Periodontal flap surgery			4 quadrants	4 quadrants	4 quadrants
Guided bone regeneration				X	X
Dental implants					X
Conservative restorative treatment	X	X			
Prosthetic restorative treatment			X		X
Professional maintenance	X	X	X	X	X

◎ **Figure 1.** Illustration of clinical information presented in case 5.



included, if present (Figure 1). As depicted in Table 1, the cases were covering a broad range of periodontal pathology. Case 1 presented a plaque-related gingivitis; case 2 a chronic moderate periodontitis; case 3 a chronic adult periodontitis; and cases 4 and 5 displayed an aggressive periodontitis with a generalised and localised nature respectively. The five related cases allowed to raise pertinent questions related to decision making in daily clinical practice. These included: 1) diagnostics in periodontal therapy/treatment approaches, 2) use of chemical plaque control agents, use of additional microbiologic or genetic diagnostic tests, 3) when is scaling and root planning followed by surgical pocket elimination, 4) use of regenerative techniques and biomaterials, 5) extraction therapy, 6) maintenance and recall intervals and 7) restorative treatments.

In response to the research questions two questionnaires were developed and administered during the short-term continuing professional development periodontal course five times consecutively at Malmö University between 2011-2014. A pre-course questionnaire was distributed at the onset of the course, containing 11 items. Four items gauged the participants' characteristics (age, clinical expertise, clinical experience and expectations). Additionally, 7 items were introduced to evaluate the participants' perceptions on the feasibility (access to cases on the internet and time spent), case-based approach (difficulty of cases and efficiency as a learning strategy) and the evolved learning goals as a result of the case-based preparation. A post-course questionnaire was presented at the end of the course. It included 10 items and gauged participants' experiences of the case-based approach during the course. Items in both questionnaires contained open ended questions and statements to be rated on a 5-point scale (1=totally disagree, 5=totally agree). Both questionnaires were screened by two periodontal experts and one educational expert on clarity and ambiguity of wording and adjusted when needed. To allow the linkage between the data in the pre- and post-course situation, the questionnaires were not anonymous. The participants provided their consent by returning the questionnaire. After the data was put into de database, the identity of the participants was removed prior to the start of the analysis.

Descriptive statistics were used to analyse the participants' reactions to the questionnaires. To investigate the effect of experience and preparation time on perceptions, participants were labelled as experienced (≥ 10 years of experience) or inexperienced (< 10 years of experience) and extensively prepared (≥ 60 minutes for five cases) or shortly prepared (< 60 minutes for five cases). Differences in perceptions between these groups and clinical expertise (dentist

Table 2. Overview of participants in each course, gender and profession.

Participants	Total participants	Gender		Profession	
		Male	Female	Dentist	Dent Hyg
Course 1	25	4	21	8	17
Course 2	25	4	21	12	13
Course 3	13	1	12	3	10
Course 4	24	2	22	5	19
Course 5	30	5	25	11	19
Total	117	16	101	39	78

Table 3. Overview of participants' characteristics (Mean, Standard Deviation, Minimum and Maximum) concerning age, experience and preparation time.

Participants	Mean	SD	Min	Max
Age (year)	44	12	23	70
Experience (year)	14	11	1	42
Preparation time (minutes)	62	42	2	190

Table 4. Participants' pre-course perceptions about the preparation strategy concerning delivery of cases, case difficulty and the efficiency on a 5-point scale from totally disagree to totally agree. Results display the amount of responses (%), mean and standard deviation.

	1.Totally disagree	2.Disagree	3.Neutral	4.Agree	5.Totally agree	Mean	SD
1. It was easy to access the cases online	6 (5.2)	4 (3.5)	6 (5.2)	22 (19.1)	77 (67.0)	4.39	1.09
2. The cases were difficult	4 (3.4)	4 (3.4)	55 (47.0)	47 (40.6)	3 (2.6)	3.36	0.76
3. I could answer to all questions in the cases	6 (5.5)	18 (16.4)	63 (53.8)	14 (12.0)	9 (7.7)	3.02	0.92
4. Solving cases to prepare the lecture was time well spent	2 (1.9)	11 (10.2)	24 (22.2)	41 (38.0)	30 (27.8)	3.80	1.02
5. I believe to be sufficiently prepared by the cases to efficiently follow the lecture	0 (0.0)	16 (14.4)	32 (28.8)	45 (40.5)	18 (16.2)	3.59	0.93

Table 5. Participants' end-course perceptions about the efficiency of case-based learning on a 5-point scale from totally disagree to totally agree. Results display the amount of responses (%) , mean and standard deviation.

Statement	1.Totally disagree	2.Disagree	3.Neutral	4.Agree	5.Totally agree	Mean	SD
1. The cases and lecture were attuned to each other	0 (0.0)	2 (1.8)	7 (6.3)	38 (33.9)	65 (58.0)	4.48	0.70
2. Solving cases before a lecture is beneficial to follow and understand the lecture	2 (1.8)	12 (10.8)	17 (15.3)	39 (35.1)	41 (36.9)	3.95	1.05
3. The approach of solving cases in combination with a lecture is an efficient method of learning	0 (0.0)	2 (1.8)	10 (8.9)	33 (29.5)	67 (59.8)	4.47	0.74
4. The use of cases is advantageous for relating theory to clinical practice	0 (0.0)	10 (9.0)	15 (13.5)	51 (45.9)	35 (31.5)	4.00	0.91
5. I experienced case solving in preparation of a lecture as positive	2 (1.8)	7 (6.4)	15 (13.6)	50 (45.5)	36 (32.7)	4.01	0.94
6. I prefer the combination of case-solving and lectures to lectures without preparation	3 (2.7)	5 (4.5)	19 (17.1)	41 (36.9)	43 (38.7)	4.05	0.99
7. The course has fulfilled my expectations	1 (0.9)	10 (8.9)	15 (13.4)	32 (28.6)	54 (48.2)	4.14	1.02
8. I perceived the case-solving approach as enjoyable	4 (3.6)	8 (7.3)	22 (20.0)	42 (38.2)	34 (30.9)	3.85	1.06
9. I believe to be sufficiently prepared by the cases to efficiently follow the lecture	1 (0.9)	8 (7.2)	23 (20.7)	50 (45.0)	29 (26.1)	3.88	0.91
10. During the lecture I could discuss my personal thoughts about the cases	4 (3.6)	6 (5.4)	22 (19.6)	42 (37.5)	38 (33.9)	3.93	1.04

vs. dental hygienist) were analysed by a one-way ANOVA (6). A paired samples t-test was used to investigate possible change in perceived level of preparation by cases before and after the course (6). The individual reported expectations and learning goals were grouped into larger categories and analysed using descriptive statistics and cross-tabulations to identify possible tendencies caused by clinical expertise, clinical experience or preparation time. All statistics were executed in SPSS 22.0 (IBM SPSS Statistics for Windows, Armonk, NY: IBM Corp.) with with a pre-set significance level of $p \leq 0.05$.

Results

In total, 119 oral health care providers participated in 5 courses between 2011-2014. The pre-course questionnaire was answered by 117 participants (response rate=98%) and the post-course questionnaire was returned by 111 participants (response rate=93%). An overview of the participants in the course is depicted in Table 2. Characteristics concerning age, experience and reported preparation time are presented in Table 3. A part of the returned questionnaires contained items that were not answered. Those items were identified as missing values.

Participants were positive about the use of internet to distribute the cases prior to the course, 86.1%

Table 6. The influence of clinical expertise (dentists and dental hygienists), experience (<10 years and ≥10 years) and pre-course preparation time (<60 minutes and ≥60 minutes) on the participants' reactions to the statements in the pre-course and end-course questionnaire. Pre-course and end-course statement numbers refer to the statements in table 3 and 4 respectively. Furthermore the M(ean) of each subgroup is displayed in combination with the characteristics of the one-way ANOVA analysis (F-value and significance) to evaluate the differences. Only significant differences between the subgroups are presented.

Statement	M	M	F	Sig.
Clinical expertise	**Dentist**	**D Hyg**		
End-course 1: The cases and lecture were attuned to each other	4.26	4.59	5.668	0.019
End-course 2: Solving cases before a lecture is beneficial to follow and understand the lecture	3.65	4.11	4.793	0.031
End-course 3: The approach of solving cases in combination with a lecture is an efficient method of learning	4.16	4.63	11.236	0.001
End-course 5: I experienced case solving in preparation of a lecture as positive	3.73	4.17	5.469	0.021
Clinical experience	**Exp<10**	**Exp≥10**		
Pre-course 4: Solving cases to prepare the lecture was time well spent	4.02	3.62	4.179	0.043
End-course 1: The cases and lecture were attuned to each other	4.64	4.35	4.748	0.032
End-course 3: The approach of solving cases in combination with a lecture is an efficient method of learning	4.68	4.30	7.537	0.007
End-course 4: The use of cases is advantageous for relating theory to clinical practice	4.23	3.84	5.271	0.024
End-course 5: I experienced case solving in preparation of a lecture as positive	4.26	3.85	5.096	0.026
End-course 7: The course has fulfilled my expectations	4.40	3.92	6.300	0.014
Preparation time	**Prep<60**	**Prep≥60**		
Pre-course 2: The cases were difficult	3.20	3.60	6.893	0.010
End-course 2: Solving cases before a lecture is beneficial to follow and understand the lecture	3.70	4.35	8.346	0.005

(totally) agreed. Concerning the case difficulty and the ability to answer the questions in the cases, participants were indecisive as respectively 47% and 53.8% answered neutral. The use of cases as an efficient preparation strategy was considered positive. A total of 65.8% and 56.7% of the participants (totally) agreed that the case preparation strategy was time well spend and felt that they were sufficiently prepared to follow the course respectively. Nevertheless 22.2% and 28.8% answered neutral. A detailed overview of the pre-course questionnaire results is presented in Table 4.

Participants' post-course reactions to the statements about the use of clinical cases (before and during) the lecture and perceived instructiveness were in general positive. Participants agreed that cases and course were attuned, cases were an efficient preparation strategy, linked theory to practice, provided the opportunity to discuss personal thoughts. Furthermore this approach to education was considered as positive, enjoyable, fulfilling expectations and was preferred to a course without cases. The participants that (totally) agreed with this statements ranged between 69.1% and 91.9%. In addition, the statements about the enjoyment of case-solving, the feeling of being sufficiently prepared by the cases and the ability to discuss personal thoughts was perceived as indecisive by respectively 20.0%, 20.7% and 19.6% of the participants. Details of the post-course questionnaire are presented in Table 5.

One-way ANOVA analysis identified multiple significant differences in perceptions between dentists and dental hygienists, experienced and inexperienced oral health care clinicians and participants with a short and extensive preparation time. Dental hygienists did significantly agree more with the statements about the connection between the cases and lecture, that case solving is beneficial to follow the lecture, the efficiency of the case-solving as a learning method and case-solving as a positive experience. Participants with less than 10 year experience significantly agreed more with the statements about case-solving to be time well spent, connection between cases and lectures, instructiveness of case-solving, case to be advantageous for relating theory to practice, case solving as a positive experience and the fulfilment of expectations. Participants with a reported preparation time above 60 minutes

◎ **Table 7.** Summary of learning goal themes (=frequency) and characteristic quotes by dentists and dental hygienists at the onset of a short-term continuing professional development periodontal course.

Learning goal	Characteristic quote
Therapy	
Treatment planning (35)	Periodontal treatment after surgery?
Extraction (27)	When to extract / try to preserve a teeth?
Surgery (27)	Surgery or proceed with the treatment
Maintenance (15)	Maintenance how often?
Initial therapy (5)	Alternatives for initial therapy
Radiography (1)	When to extract? Could indications be defined directly on a radiograph?
Full mouth treatment (1)	Full-mouth?
Medication (7) – Antibiotics (7)	What is the influence of medication on periodontitis?
Immunological aspects (3)	Immunological aspects in periodontology
Periodontology and oral implants (9)	How to place implants in patients with periodontal disease?
Biomaterials (5)	The effects biomaterial treatment in deep pockets/severe periodontitis
Prosthetics (12)	As a dental hygienist I have no idea of prosthetics
Instruments (US-Manual) (4)	Manual vs. ultrasonic instruments
Periodontitis (16)/Peri-implantitis (7)	Juvenile periodontitis?
Orthodontics (1)	Is it possible to start orthodontics after periodontal treatment?
Furcation (2)	Should teeth with furcations be operated?
Collaboration/Referral	
Collaboration dentist-dental hygienist (8)	When does the dental hygienist perform the treatment?
Collaboration in a dental team (2)	Who does what within a team?
Referral (25)	Which patients should I refer and which not?
Compliance	
Motivation of patients (25)	non-compliant patient, what can you do to convince the patient?
Patients at risk	
Patients with risk factors (9)	Risk assessment in patients with periodontitis
Prognosis (8)	What sort of patients has a bad prognosis?
Smoking (5)	Should smokers be forced to stop because it's good for them?

significantly agreed more with the statements that cases were difficult and that case solving was beneficial to understand the lecture. Furthermore they also significantly agreed more with the statements that case solving is an efficient method of learning, that cases are advantageous to relate theory to practice, that case solving is enjoyable and that they felt sufficiently prepared by the cases. Details of significant results are presented in Table 6.

Repeated measures t-test analysed the participants' reactions to the statement: "I believe to be sufficiently prepared by the cases to efficiently follow the lecture" in both the pre- and post-course questionnaire. It revealed that the participants valued the level of preparedness by clinical cases significantly higher after the course than before ($M_{pre\text{-}course}$=3.58, $M_{end\text{-}course}$=3.91, t=-3.333, p=0.001). The reported means differ slightly from those reported in tables 4 and 5, as the repeated measures t-test only included the participants that answered both questions.

Reported expectations could be labelled as refreshing knowledge (n=34), acquiring new knowledge (n=50) and mastering the course subject (n=30). Furthermore specific expectations were identified, including the need for discussion about treatment planning (n=30), risk factors and prognosis (n=15), collaboration with colleagues and referral strategy of patients (n=4) and maintenance (n=2).

Participants were also asked to provide three learning goals. After an initial analysis, five broad categories could be identified, including therapy, collaboration and referral, compliance, patients at risk and other. Table 7 provides a summary of all reported individual learning goal themes with quotes to highlight the common thoughts of the participating dentists and dental hygienists.

No tendencies in expectations and reported individual learning goals could be identified based on the participants' profession, experience or preparation time.

Discussion

Findings suggest that introducing clinical cases in a short-term continuing professional development periodontal course as an active learning strategy has an acceptable feasibility and is positively perceived by the participants with pre-course expectations ranging from brushing up and acquiring new knowledge to mastery of the course subject. The most frequently reported individual learning goals are related with treatment planning, the choice between treatment, extraction and surgery, referral and motivation of patients.

The use of clinical cases has been promoted as an active learning strategy in support of expert practice in dentistry (8). Participants in the present study highly appreciated this educational approach although it required some additional preparation time before the course. These positive perceptions are in line with findings of McKenzie (15) and Koole et al. (12) about case discussions in small groups in undergraduate education. In the present study, however, pre-course clinical cases were considered individually, although multiple participants reported that they had discussed the cases in advance with their colleagues in their clinical practices.

Whereas the traditional format of lectures requires no preparation, participants in the present study had to invest time to go through the clinical cases. Findings suggest that the Swedish dentists and dental hygienists in the course were willingly to do so, as they reported to have spent on average 62 minutes. It is plausible to assume participants were driven by intrinsic motivation, due to the lack of consequences associated with non-compliance. This factor is also a plausible explanation for the reported range in spent time (2-190). Each participant in the study has a different motivation to follow the course and has a different work situation, which may have influenced the preparation time. This time investment to prepare the continuous professional development course fits the image of professionals engaging in lifelong learning and a continuous search for improvement (5).

Expectations prior to the course could be categorised as a search for information (refreshing and acquisition of knowledge) but multiple participants also reported that they wanted to master the course subject. This refers to both the theoretical background and the transfer to clinical practice. In a solely theoretical course this is remarkable. In contrast with students, most participating dentists and dental hygienists were experienced professionals. As a result, they have encountered far more clinical situations to relate to. Furthermore they follow continuous development educational courses with the intention to improve as a practitioner (4). Hence they seek support for their clinical practice. In addition, clinical cases in the course may also have facilitated a focus on a transfer from theory to practice.

The identification of the participating dentists' and dental hygienists' individual learning goals revealed a focus on therapy planning, as was expected. Participants, however, expressed also a question to clarify the indicators to decide whether a treatment should be continued or initiated, teeth should be extracted or preserved or to consider surgery and about when to refer. This was not anticipated as the larger part of the participants were experienced clinicians dealing with those choices on a daily basis. Furthermore a frequently reported learning goal was how to motivate (non-compliant) patients. The data in this study refers to a combined group of 117 dentists and dental hygienists, both involved in oral health care and seeking additional education in periodontology. As a result, the findings have to be interpreted within this context. They cannot automatically be generalised, but have to be considered from the perspective of oral professionals engaging in lifelong learning. The results provide an indication of what occupies the minds of those clinicians in their clinical practice.

Both expectations and individual learning goals reported in the pre-course questionnaire had also an alternative purpose besides the study. They enabled the lecturer to direct the course content to the specific learning needs of the participants, which may have contributed to the overall positive perception of the course.

Reflection is an important attribute for professionals to learn from their experiences and to direct their lifelong learning trajectory (17). Awareness of own competences, knowledge, experience, values, etc. , which is called a frame of reference, is important in this process, because it defines a person as a professional and controls his/her actions (11, 16). The pre-course cases may have helped to activate a participants' prior knowledge, improving the capacity to integrate new understandings (3).

Although findings suggest a positive effect of introducing clinical cases in a theoretical short-term continuing professional development periodontal course, results remain inconclusive about the actual effectiveness on learning and transfer to the clinical practice. This would require a study design

with a control group and pre- and post- testing of learning gain, which was not performed during the course and could be considered as a shortcoming of the course (2, 14). On the other hand, the absence of examination did not provoke stress or competition between participants, which could have been a confounding factor in the results. Future studies should focus on the effect of educational strategies in continuing professional education in terms of knowledge development and transfer to clinical practice.

Conclusions

Clinical cases were used as an active learning strategy to activate prior knowledge, attune participants' mind-set to the course and to synchronise theory to practice in a short-term continuous professional development periodontal course. This course format was positively perceived by the participating dentists and dental hygienists in terms of feasibility and learning potential. Furthermore individual learning goals were focussed towards daily clinical activities. These findings may suggest that integrating a case-based approach in continuing professional development course may be worthwhile to further investigate in the transition from passive attendants to active lifelong learners.

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Protein and bacteria binding to exposed root surfaces and the adjacent enamel surfaces *in vivo*

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Abstract

◎ Exposure of root surfaces due to inflammatory tissue breakdown is a clinical characteristic of periodontitis. The gingival margin may further recede during treatment. Pellicles and early dental plaque on enamel surfaces of periodontitis patients have previously been described. The binding properties of exposed root surfaces, which may affect the incorporation of proteins from especially the GCF into the enamel pellicle and thereby early dental plaque formation are largely unknown. The aim of this study was to examine if exposed root surfaces could affect pellicle and initial dental plaque formation on the enamel surface by the analysis of proteins and early adhering bacteria binding to the exposed root surfaces and to the adjacent, gingival enamel surface.

Supragingival pellicle and plaque samples were taken from exposed root surfaces and the adjacent enamel surfaces in eleven surgically treated periodontitis patients. For comparison, samples were taken from enamel surfaces of teeth not in need of treatment. Additionally, subgingival bacterial samples were taken. Pellicle proteins were analysed by SDS-PAGE, immunoblotting and image analysis, and bacterial samples by culturing.

Significantly more plasma proteins and bacteria were found on the exposed root surfaces than on the enamel. The depth of the gingival recessions was negatively correlated to the amount of plasma proteins in the enamel pellicle. *Actinomyces* spp. were most frequently found on the exposed root surfaces. The total viable counts and streptococci (%TVC) were positively correlated between subgingival samples and samples from the root surface and enamel of surgically treated teeth. A positive correlation was also found for the findings of Gram-negative anaerobes in subgingival samples and samples from the enamel surface.

Our findings suggest that an exposed root surface has binding properties different from an enamel surface and could affect early biofilm formation on the adjacent enamel surface.

Key words

Bacterial adhesion, dental enamel, dental plaque, gingival crevicular fluid, gingival recession

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Proteiner och bakterier som binder till exponerade rotytor och de angränsande emaljytorna *in vivo*

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Sammanfattning

◎ Parodontit kännetecknas av inflammatorisk vävnadsnedbrytning runt tänder som bland annat leder till blottlagda rotytor sk. retraktioner. Till följd av en behandling kan gingivalkanten dra sig ytterligare tillbaka. Pellikel och tidigt plack på emaljytor hos parodontitpatienter har tidigare beskrivits. Bindningsegenskaper av blottlagda rotytor, som kan påverka inkorporeringen av proteiner speciellt från GCF i pellikeln på emaljen och därigenom tidigt dentalt plack är i stort sett okända. Syftet med denna studie var att undersöka pellikel och tidig plackbildning på exponerade rotytor samt huruvida rotytorna påverkar inbindning av proteiner och bakterier på den angränsande emaljytan.

Supragingivala pellikel- och plackprover togs från exponerade rotytor och de angränsande emaljytorna hos elva kirurgiskt behandlade parodontitpatienter. För jämförelse togs även prover från emaljytorna på tänder som inte varit i behov av en behandling samt subgingivala bakterieprover. Pellikelproteinerna analyserades med SDS-PAGE, immunoblotting och bildanalys, och bakterieprover genom odling.

Signifikant fler plasmaproteiner och bakterier hittades på de exponerade rotytorna än på emaljen. Retraktionsdjupet var negativt korrelerat med mängden plasmaproteiner i emaljpellikel. *Actinomyces* spp. hittades oftast på de exponerade rotytorna. Totalantalet bakterier och antal streptokocker (i % totalantal) var positivt korrelerade mellan subgingivala prover och prover från rotytan och emalj av kirurgiskt behandlade tänder. En positiv korrelation noterades även för gramnegativa anaerober i subgingivala prover och prover från emaljytan.

Våra resultat tyder på att en exponerad rotyta har bindningsegenskaper som skiljer sig från en emaljyta och kan påverka pellikel och tidig plackbildning på den angränsande emaljytan.

Introduction

Chronic periodontitis is a bacterial infectious disease characterized by inflammation and eventual breakdown of the tissues surrounding the teeth. Gingival recessions exposing root surfaces are frequently seen (35). Other clinical signs of periodontitis are increased probing depths, bleeding on probing and gingival crevice fluid (GCF) flow (4, 38). On running out of the gingival crevice the GCF, a plasma derivate, moistens the part of the tooth surface that is in contact with the gingival margin. Like others, we previously have demonstrated that typical plasma proteins can bind to hydroxyapatite *in vitro* and promote the adherence of bacteria (1, 5, 10, 41). It was further demonstrated that they were incorporated into the dental pellicle on enamel surfaces *in vivo* (10, 19, 32, 33, 36). As the GCF flow varied due to periodontal inflammation so did - by and large - the amount of plasma proteins in the dental enamel pellicles. However, some of these plasma proteins did not seem to follow this pattern showing lower amounts of fibronectin and fibrinogen in periodontitis patients (33) than in periodontally healthy patients (32). Gingival recessions and exposed root surfaces may explain this observation as the distance from the gingival margin to the enamel surface increases. To what extent the presence of gingival recessions may further affect the microbial composition of supragingival plaque has only rarely been investigated (15). The primary aim of this study was to examine the effect of an exposed root surface on pellicle formation and early bacterial binding on the adjacent enamel surface. Selected pellicle proteins and bacteria in early dental plaque formed on these surfaces *in vivo* on teeth in periodontitis patients, were therefore examined.

Materials and Methods

Subjects and sampling principles

11 periodontitis patients (age 46 to 58 years, 7 males, 4 females) with exposed root surfaces after periodontal flap surgery were included into the study. They were diagnosed with general chronic periodontitis (3) in a remaining dentition of 23 to 28 teeth and recruited at the Specialist Dental Care Centre, Malmö, Sweden, upon completion of comprehensive periodontal therapy (non-surgical and surgical periodontal therapy). General exclusion criteria were medications or medical conditions affecting oral health and use of antibiotics during six months prior to the study. Ten of the included patients were smokers. Good oral hygiene (dichotomous PlI < 15%) was a prerequisite for participation. Surgically treated teeth with radiographic bone loss of > 1/3 of the root lengths were selected for sampling. Presurgical probing depths were ≥ 5 mm with bleeding upon probing (surgically treated teeth). Teeth that had not been in need of any periodontal treatment and having radiographic bone loss of ≤ 1/3 of the root lengths, probing depths of no more than 4 mm and no bleeding on probing (non-treated teeth) were also selected.

The pairwise surgically treated and non-treated teeth were of the same tooth type and were selected in such a way that they were either positioned contralaterally or in the opposite jaw. Teeth with buccal or lingual restorations were excluded. No furcation-involved tooth was included. Clinical characteristics as measured at enrollment into the study are given in Table 1. Probing depth in mm and bleeding on probing (dichotomous) were registered at four sites per tooth (mesial, buccal, distal, lingual). The recession depth per tooth was calculated as the average of the

Table 1. Clinical characteristics at the teeth selected for pellicle and plaque sampling as mean of individual mean values for the surgically treated and non-treated teeth (mean and range; n = 11).

	Surgically treated teeth	Non-treated teeth
Pockets of 4 mm*	12 (0 - 31)	4 (0 - 25)‡
Pockets of 5 - 6 mm*	1.1 (0 - 6)	0
Bleeding upon probing*	8 (0 - 25)	5 (0 - 19)
Recession depth†	1.54 (0.61 - 2.67)	0.48 (0.03 - 1.9)§

*percentage of sites (4 sites/tooth)

†distance from the gingival margin to the CEJ in mm (6 sites/tooth)

‡p<0.05 and §p<0.01 for differences between non-treated and surgically treated teeth

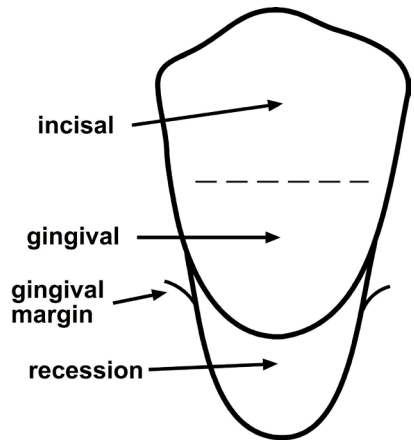
distance from the gingival margin to the CEJ in mm measured at six sites per tooth (mesiobuccal, buccal, distobuccal, distolingual, lingual, mesiolingual) at both surgically treated and non-treated teeth.

Pellicle and supragingival plaque samples were taken from exposed root surfaces with a recession depth of at least 3 mm (surgically treated teeth only). Additional supragingival samples were taken from the enamel surfaces of the gingival and incisal parts of the crown of all selected teeth (see Figure 1). The root surface areas from which samples were taken were smaller than either of the other supragingival sampling areas. Care was taken to always keep a safety distance of around 1 mm to the gingival margin while samples were taken. Therefore, only exposed root surfaces with a recession depth of at least 3 mm were suitable for sampling, and sampling from exposed root surfaces of the non-treated teeth was not possible. Subgingival bacterial samples were taken from both the surgically treated and the non-treated teeth. The study protocol was approved by the Regional Ethical Review Board in Lund/Sweden (Dnr 2010/377).

Pellicle collection and analysis

To create healthy conditions in the gingival margins, all teeth were professionally cleaned every other day for a 2-week period. The dental pellicles were collected and analysed as previously described (9).

Figure 1. Supragingival sampling from a surgically treated tooth. Pellicle and supragingival plaque samples were taken from exposed root surfaces at gingival recessions with an extension of at least 3 mm. Additional supragingival samples were taken from the enamel surfaces of the gingival and incisal parts of the crown. **Supragingival sampling from a non-treated tooth.** As there were practically no recessions present at the non-treated teeth pellicle and supragingival plaque samples were taken only from the enamel surfaces of the gingival and incisal parts of the crown.



In brief, pellicle formation was allowed for 60 min after polishing of the teeth using a rubber cup and fine-grade pumice (Lapis pumicis pulveratus subtillissimus, APL, Kungens Kurva, Sweden). After rinsing with water the selected teeth were isolated with cotton rolls and air-dried. Pellicles were collected from the exposed root surfaces and separately from areas of the gingival and incisal parts of the buccal and lingual enamel surfaces, respectively (see Figure 1), by alternately rubbing the surface with fibre-pellets (Quick-sticks™, Dentonova AB, Huddinge, Sweden) soaked in 2% SDS-solution, and dry pellets. All fibre-pellets from the gingival and incisal enamel surfaces and the exposed root surfaces, respectively, were pooled from all surgically treated teeth (three samples) and from all non-treated teeth (two samples) in each patient and, thus, five individual samples per patient were forwarded for analysis. In the analysis, the pellicle proteins were de-solved from the fibre-pellets by heat treatment in SDS-PAGE sample buffer and recovered by centrifugation. The samples were run on pre-cast 4-15% gradient gels, silver-stained or blotted onto nitrocellulose membranes using the Pharmacia automated Phast-System® according to the manufacturer's description and recommendations (Pharmacia/LKB, Biotechnology, Stockholm, Sweden).

As in our previous studies (10, 32, 33), a panel of antibodies (Sigma-Aldrich Sweden AB, Stockholm, Sweden, except for antibody to PRP which was kindly provided by Dr Bennick, University of Toronto, Canada) to IgA and typical plasma (IgG, albumin, fibronectin and fibrinogen) and typical salivary proteins (amylase and proline-rich proteins (PRP)) was used to identify specific proteins by immunoblotting. As staining intensity could vary between different gels and different immunoblot membranes, samples to be compared were run on the same gel. The detection limits for all proteins had previously been determined from the staining of purified, serially diluted proteins in the immunoblots (32).

Stained gels and membranes were scanned and digitized prior to semi-quantification of the proteins by densitometric analysis (ImageJ 1.40g, public domain program; NIH).

Plaque sampling

The selected teeth were polished using pumice as above, and plaque was allowed to form for 4 h, undisturbed from food and fluid except water. The teeth were rinsed with water, isolated with cotton rolls and air-dried prior to collection of plaque by

scraping the respective tooth surfaces with sterile scalers (Scaler N°2 REF 418 04, Produits Dentaires S.A. Vevey 1800 Switzerland).

Supragingival samples from the exposed root surfaces of the surgically treated teeth and from the gingival and incisal enamel surfaces of the surgically treated and non-treated teeth were collected in VMGA III transport media. The subgingival samples were immediately thereafter obtained from the mesial aspects of each non-treated tooth and from a surgically treated approximal site of each surgically treated tooth. Two sterile paper points size 40 (Top Dent, DAB Dental Upplands Väsby, Sweden) were inserted into the gingival sulcus for 10 s and then transferred into the VMGA III transport media (11, 22).

Bacterial examination

Plaque samples were vortexed prior to serial dilution in peptone water (50 mM K-phosphate buffer, 0.4% KCl, pH 7). Diluted samples were plated on MS (Mitis salivarius) agar for growth of *Streptococcus* spp. and on CFAT (Cadmium sulfate-fluoride-acridine trypticase) agar (46) for growth of *Actinomyces* spp., which were further characterized by their rod-shaped or filamentous appearance after Gram-staining. Samples were cultured on TSBV (Trypticase soy bacitracin vancomycin) agar (37) for growth of *Aggregatibacter actinomycetemcomitans* and on Brucella agar (enriched with 5% horseblood, 0.5% hemolyzed horseblood and 5mg/l menadione) for determination of the total viable counts (TVC), and numbers of black-pigmented *Prevotella* spp., *Porphyromonas gingivalis*, *Capnocytophaga* spp., *Campylobacter rectus* and *Fusobacterium nucleatum* from their typical morphological appearance (24). *P. gingivalis* was distinguished from *Prevotella* spp. by testing autofluorescence. The MS agar plates were incubated for 3 days and the TSBV and CFAT agar plates for 5 days in 90% N_2 and 10% CO_2 at 37°C. The Brucella-agar plates were incubated using the hydrogen combustion technique (95% H_2 and 5% CO_2 at 37°C) for 7 days (23).

Gingival crevice fluid (GCF) flow

To estimate the inflammatory status of the sample sites, the GCF flow was measured using PerioPaper Strips® and a Periotron 8000® device (Oraflow, Smithtown, NY, USA). The Periotron value was transformed into a fluid volume (µl) using a standard curve generated by calibration with standardized fluid volumes.

Statistics

Apart from descriptive statistics, Wilcoxon matched pairs signed rank sum test was applied for comparative tests as well as the Spearman correlation test. A number of eleven subjects allows for non-parametric statistical analysis. IMB SPSS Statistics 20 was used.

Results

Pellicle composition

IgG, albumin, amylase and IgA were detected in 90% to 100 % of the samples. Fibrinogen and fibronectin were identified to the highest extent on the exposed

Table 2. Detection frequencies* of proteins in gingival and incisal pellicles formed on surgically treated and non-treated teeth.

	Exposed root surfaces	Gingival pellicle		Incisal pellicle	
		Surgically treated teeth	Non-treated teeth	Surgically treated teeth	Non-treated teeth
IgG	100	100	100	100	100
Albumin	100	100	100	100	100
Fibronectin	90.9	63.6	54.5	54.5	45.5
Fibrinogen	81.8	72.7	45.5	63.6	54.5
Amylase	90.9	90.9	90.9	100	100
IgA	90.9	100	90.9	90.9	100
PRP	54.5	36.4	45.5	45.5	36.4

*percentage of the samples where the protein was detected

root surface and on the gingival enamel of surgically treated teeth, and especially on the former (80 to 90%; Table 2). With one exception for fibrinogen, they, like PRP, were otherwise found in ≤ 55% of all samples.

Semiquantitative amounts of total protein (from silver-stained gels) and specific proteins were obtained from the pixel values (Table 3). Samples taken from the exposed root surfaces of the surgically treated teeth displayed the highest amounts of proteins. The amounts of total protein, plasma proteins (IgG, albumin, fibronectin and fibrinogen), and the detection frequencies of fibronectin and fibrinogen (Table 2) in the pellicles displayed an overall pattern of higher values on the surgically treated than on the non-treated teeth at both the gingival and incisal enamel surfaces.

Bacteria in supra- and subgingival biofilms

The bacterial counts per sample varied considerably and few statistically significant differences were obtained (Table 4). The mean total viable counts (TVC) on exposed root surfaces were, however, statistically significantly higher than on the adjacent gingival part of the crowns (gingival enamel) of the same teeth.

Streptococcus spp. were identified in 94% of all the respective supra- and subgingival samples.

Besides a lower mean percentage of TVC on the gingival enamel surfaces of the non-treated compared with the treated teeth, no notable differences were seen for their prevalence between the various surfaces (Table 4).

Actinomyces spp. were detected in 53% of all samples counted together and in highest numbers on the exposed root surfaces. Their total counts were notably higher on the exposed root surfaces than on the other supragingival surfaces (Table 4). Both *Actinomyces* spp. and *Streptococci* spp. were more prevalent in subgingival samples from the surgically treated compared with non-treated teeth.

Gram-negative species (*Capnocytophaga* spp., *Campylobacter rectus*, *F. nucleatum*, black-pigmented *Prevotella* spp.) were more often found on the exposed root surfaces and on the gingival enamel surfaces compared with the incisal enamel surfaces, and were present in the majority of the subgingival samples. *A. actinomycetemcomitans* or *P. gingivalis* were not detected.

GCF flow

The GCF flow was lower at non-treated (0.029 ± 0.0097 µl/10s) than at surgically treated teeth (0.045 ± 0.021 µl/10s; $p < 0.05$).

Exposed root surfaces

The recession depth (mm) was negatively correlated to the amount of the plasma pellicle proteins (pixel values) in the gingival enamel pellicles. In the case of albumin and fibronectin on surgically treated teeth, this correlation reached statistical significance (Table 5).

Negative correlation coefficients were also observed for root surface exposure and the salivary proteins (-0.18 to -0.36) without reaching statistical significance.

Correlation supra- and subgingival plaque

We found a positive and statistically significant correlation between bacterial findings of TVC, streptococci and Gram-negative anaerobes in supragingival, enamel plaque samples and in subgingival samples at the surgically treated teeth (Table 6). TVC and streptococci were similarly correlated between the subgingival samples and exposed root surfaces. The subgingival and supragingival samples from the non-treated teeth did not correlate in the same manner.

Discussion

In the present study, dental pellicles and early dental plaque were formed on exposed root surfaces and on enamel surfaces under the influence of a shallow gingival sulcus created by surgical pocket elimination and on the enamel surfaces influenced by a pristine shallow gingival sulcus at non-treated teeth.

Semi-quantification of the amount of plasma proteins in the pellicle revealed values in the order: exposed root surfaces > surgically treated teeth > non-treated teeth. The highest values for plasma proteins on the root surfaces, despite smaller areas sampled, were paralleled by a negative correlation between the amount of protein in the enamel pellicle and the depths of the recessions. This suggests that the amount of plasma proteins from the GCF reaching the enamel surface could be reduced through protein binding to the exposed root surface with a higher surface roughness compared with enamel (44). The exposed root surfaces would mainly be a dentin surface as the root cementum usually is thin in the coronal part of the root (8) and can be removed by abrasive tooth brushing (34). A more frequent presence of gingival recessions and, thus, of protein-binding exposed root surfaces in peri-

Table 3. Amounts of protein (pixel values) and ratios thereof in pellicles formed on exposed root surfaces of surgically treated teeth and on gingival and incisal enamel surfaces of surgically treated and of non-treated teeth. Pixel values (mean ± SD) obtained from image analysis of the silver-stained gels and immunoblots.

	Exposed root surfaces		Gingival pellicle		Incisal pellicle	
			Surgically treated teeth	Non-treated teeth	Surgically treated teeth	Non-treated teeth
Total proteins a	6439 ± 1873		3743 ± 2610	2976 ± 1771	3055 ± 1694	2690 ± 2042
		1.7†		1.3	1.1	
IgG	5180 ± 3083		2928 ± 3426	2057 ± 1458	1625 ±1777	1104 ± 703
		1.8†		1.4	1.5†	
Albumin	8100 ± 2627		5203 ± 3419	3966 ± 2686	2445 ± 2657	1543 ± 986
		1.6		1.3	1.6†	
Fibronectin	4609 ± 3541		2469 ± 3655	1061 ± 1464	1116 ± 1694	448 ± 845
		1.9†		2.3	2.5	
Fibrinogen	4338 ± 3493		1217 ± 1495	732 ± 1260	893 ± 930	435 ± 682
		3.6		1.7†	2.1	
Amylase	4272 ± 3128		2275 ± 1602	1882 ± 1674	2603 ± 1585	2277 ± 1811
		1.9		1.2†	1.1	
IgA	5237 ± 2715		3224 ± 3213	1636 ± 1316	2793 ± 3074	2009 ± 2598
		1.6		2.0	1.3	
PRP	1789 ± 2445		454 ± 962	835 ± 1441	955 ± 1656	419 ± 934
		3.9		0.5	2.3	

*total amount of proteins as determined from silver-stained gels †$p < 0.05$ for the difference represented by the ratio

Table 4. Bacteria in early supragingival plaque from gingival and incisal parts of the crowns (enamel surfaces) and exposed root surfaces (surgically treated teeth only) and in subgingival samples from surgically treated and non-treated teeth (mean ± SD; n = 11).

	Supragingival plaque					Subgingival samples	
	Exposed root surface	Gingival plaque		Incisal plaque		Surgically treated teeth	Non-treated teeth
		Surgically treated teeth	Non-treated teeth	Surgically treated teeth	Non-treated teeth		
log (TVC*)	4.66 ± 1.25¶	3.31 ± 1.34	4.10 ± 1.75	3.16 ± 0.99	3.39 ± 1.63	6.31 ± 0.75	5.92 ± 0.69
Streptococcus spp.†	66.8 ± 70.9	73.7 ± 43.3	45.1 ± 40.1	61.6 ± 38.3	72.8 ± 58.0	71.3 ± 35.5	58.3 ± 54.1
Actinomyces spp.‡	3844 ± 10962	17.3 ± 36.1	25.4 ± 60.0	7.3 ± 12.7	10 ± 18.4	2765 ±4 651	1105 ± 1477
Actinomyces spp.§	72.7%	36.4%	36.4%	36.4%	27.3%	90.9%	72.7%
Gram-negativ species‖	27.3%	27.3%	27.3%	18.2%	18.2%	81.8%	81.8%

*decadic logarithm of the total viable counts
†percentage of TVC
‡total counts
§detection frequency
‖C. *rectus, Capnocytophaga* spp., F. *nucleatum*, black-pigmented *Prevotella* spp.
¶p < 0.01 plaque on the exposed root surface vs gingival enamel surface of surgically treated teeth

odontitis patients compared to periodontal healthy subjects (35) may explain our previous findings of a low prevalence of fibronectin and fibrinogen in dental pellicles formed on teeth in periodontitis patients (33) compared with pellicles formed during experimental gingivitis in periodontally healthy individuals (32).

In the present study, overall higher values for total and specific proteins were found on the enamel of the surgically treated teeth compared with non-treated teeth (Table 3), despite more exposed root surfaces in the treated ones. The parallel clinical findings of greater probing depths, more bleeding on probing and a higher GCF flow at the surgically treated sites compared to the non-treated sites, suggest that the difference may result from a remaining albeit substantially reduced infiltrated connective tissue after periodontal surgery (45) whereas the non-treated sites, i.e. healthy sites, never had been exposed to the influence of a subgingival infiltrated connective tissue. The result further suggests that a higher GCF flow could overcome the effect of an exposed root surface.

Streptococci were prevalent in all types of samples. In corroboration with the present study, Streptococci and *Actinomyces* spp. were previously identified on exposed root surfaces (12, 13) and *Actinomyces* spp. were more frequently found on the exposed root surfaces than on the enamel surfaces (14). Our findings of more *Actinomyces* spp. and a higher TVC in subgingival than in supragingival samples are in contrast to previous findings (33). This may be due to the fact that the supragingival plaque was sampled from the gingival part of the enamel surface only, and within four hours after a dental prophylaxis, whereas the subgingival samples – which were taken and analysed solely in their function as a possible source for the formation of the supragingival plaque - was formed during a longer period of time. The bacterial numbers in the very early stages of su-

Table 5. Correlation between the recession depth (mm) and the amount of total and specific proteins (pixel values) in the pellicles of the gingival enamel surfaces (Spearman correlation coefficient).

Gingival pellicle proteins	Recession depth at	
	surgically treated teeth	non-treated teeth
Total protein	-0.498	0.027
IgG	-0.504	-0.525
Albumin	-0.677*	-0.58
Fibronectin	-0.619*	-0.501
Fibrinogen	-0.401	-0.396

*p<0.05

Table 6. Correlation between the bacterial findings in subgingival and supragingival samples from surgically treated and non-treated teeth (Spearman correlation coefficient).

	Surgically treated teeth		Non-treated teeth
	Exposed root surfaces	Gingival enamel surfaces of the crowns	Gingival enamel surfaces of the crowns
TVC	0.785‡	0.794‡	0.157
Streptococcus spp. *	0.805‡	0.788‡	-0.263
Actinomyces spp.†	-0.254	0.126	-0.273
Gram-negative species†	0.082	0.731§	0.214

*%TVC
†frequency distribution
‡p<0.01
§p<0.05

pragingival plaque formation must be expected to be lower than in subgingival samples formed during a longer period of time even if the bacterial numbers in the periodontal pocket could be reduced by the dental prophylaxes (43). Another explanation could be the difference in sample collection in the present study where a curette was used for supragingival sampling and paper points for the subgingival samples.

Four gram-negative taxa (*Capnocytophaga* spp., *Campylobacter rectus*, *F. nucleatum*, black-pigmented *Prevotella* spp.) were identified in the supragingival samples. These species were previously identified shortly after tooth cleaning and are found to increase over time in growing supragingival plaque (30, 39). This may indicate that the clean supragingival environment attained directly after a dental prophylaxis can provide a favourable surrounding for the adhesion of Gram-negative species. Gram-negative species in extra-crevicular intraoral sites (20) may contribute to their supragingival colonization and especially on the incisal part of the crown where they were also identified in the present study.

Early appearance of Gram-negative bacteria in the dental plaque is supported by previous findings of *F. nucleatum* and *P. gingivalis* binding to saliva and plasma components like fibrinogen, fibronectin and albumin, identified in the *in vivo* pellicles (6, 17, 26, 32, 33).

In the present study, a time period of 60 min was used for pellicle formation and four hours for early plaque formation. It was reported that the pellicle composition and structure changes during the first two hours of formation due to e.g. replacement and proteolytic degradation of proteins and protein-protein interactions resulting in more globular macromolecule structures over time (16, 18). After 24 hours, a thick layer with adhering bacteria covering the pellicle was seen (16). In a recent study (18), proteomic analyses suggested that the majority of the identified components in the two-hour pellicle were present already after one hour and, interestingly, more components were derived from serum *via* the gingival crevice than from saliva. Studies further show that no bacteria except for some streptococci may appear before one to two hours after a tooth has been polished (21). Thus, a one-hour pellicle would be at the state when bacterial adhesion starts. Binding to pellicle components is also most plausibly of importance for the adherence of bacteria found in the early four hours plaque even if inter-bacterial binding occur (27).

The positive and statistically significant correlation between bacterial findings in supragingival (exposed root surface and gingival part of the crown) and subgingival samples at the surgically treated teeth (Table 6) corroborates a previous report (42). The number of Gram-negative bacteria on the exposed root surfaces was, however, not correlated to their numbers in the subgingival samples. This may be explained by different surface characteristics resulting in different adherence properties of the pellicles on the exposed root surfaces compared with the enamel surfaces.

Smokers have evidence of more severe periodontal disease than non smokers and are overrepresented in patients referred for the treatment of periodontal disease (15, 28). As far as the impact of smoking on flow and content of the GCF is concerned, data are, however, not consistent (25, 40). The GCF flow reported in this study was within the range of data previously reported for both smokers and non-smokers after treatment (2). It may thus be anticipated that the results of this study apply irrespective of smoking habits.

In periodontal maintenance patients, recurrent periodontal disease and root caries are the most frequent reasons for tooth loss (31). Tooth loss in this category of patients is significantly associated with the degree of initial periodontal bone loss often coinciding with exposed root surfaces (7, 29). Thus, knowledge of the mechanisms in biofilm formation on exposed root surfaces – from a clinical view point – may be of significance.

Our data suggest that exposed root surfaces may affect pellicle and early dental plaque formation on the enamel surface. Direct contact to the GCF oozing from the gingival margin and close proximity to the subgingival microflora seem to be involved in this process.

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Declaration of interest:

The authors report no conflicts of interest.

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Oral health and oromotor function in rare diseases – a database study

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Abstract

◎ The aim was to study oral health and oromotor function in individuals with rare diseases. A disease is defined as rare when it affects no more than 100 individuals per million population and leads to a marked degree of disability. An affected nervous or musculoskeletal system, cognitive impairment, neuropsychiatric disorders and craniofacial malformations are common in rare diseases and may all be risk factors for oral health and oromotor function.

In 1996-2008, 1,703 individuals with 169 rare diseases, aged 3-67 years, answered a questionnaire about general health, oral health and orofacial function and 1,614 participated in a clinical examination. A control group of 135 healthy children, aged 3-14 years, was also included in the study. Oral health was examined by a dentist and oromotor function by a speech-language pathologist. The participants with rare diseases were recruited via family programmes, referrals to the clinic and research projects, while the controls were randomly selected from a Swedish municipality.

In the diagnosis group, 40% had moderate or severe problems coping with dental treatment, 43% were receiving specialised dental care. Difficulties related to tooth brushing were common compared with the controls. Approximately two thirds of the study group and the control group were caries free. Frontal open bite, long face and high palate were common in individuals with rare diseases compared with controls. Oromotor impairment was a frequent finding (43%) and was absent among the controls. There was a significant correlation between oromotor impairment and certain structural deviations and oral-health issues.

Compared with healthy controls, individuals with rare diseases often have difficulty coping with dental treatment and managing tooth brushing. Dysmorphology and oromotor dysfunction are frequent findings in this population and they often require extra prophylactic dental care and access to specialised dental care in order to prevent oral disease.

Key words

Rare disease, disability, odontology, oral health, oromotor dysfunction

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Oral hälsa och oralmotorisk funktion hos personer med sällsynta diagnoser – en databasstudie

LOTTA SJÖGREEN, JAN ANDERSSON-NORINDER, JOHN BRATEL

Sammanfattning

◎ Syftet var att studera hur oral hälsa och oralmotorik påverkas vid sällsynta diagnoser. Dessa definieras som sjukdomar som finns hos högst 100 personer per miljon invånare och som leder till omfattande funktionsnedsättning (Socialstyrelsen).

Neurologisk, neuromuskulär och neuropsykiatrisk påverkan, kognitiv funktionsnedsättning och kraniofacial missbildning är vanligt förekommande vid sällsynta diagnoser och utgör riskfaktorer för oral hälsa och oralmotorisk funktion.

Under perioden 1996–2008, besvarade 1703 personer med 169 olika sällsynta medicinska tillstånd, 3–67 år, ett frågeformulär om allmän hälsa, oral hälsa och orofacial funktion och 1614 deltog i en klinisk undersökning. I studien ingick också en kontrollgrupp bestående av 135 friska barn, 3–14 år. Oral hälsa undersöktes av tandläkare och den oralmotoriska bedömningen gjordes av logoped. Personer med sällsynta diagnoser rekryterades via familjevistelser, remisser och kliniska forskningsprojekt. Kontrollerna var slumpmässigt utvalda från en svensk kommundel.

I diagnosgrupperna hade 40% måttliga till svåra problem att medverka vid tandbehandling och 43% hade tillgång till specialisttandvård. Svårigheter med tandborstning var vanligt jämfört med kontrollerna. Två tredjedelar av både diagnosgrupp och kontrollgrupp var kariesfria. Frontalt öppet bett, long face och hög gom var vanligt hos personer med sällsynta diagnoser i förhållande till kontrollerna. Oralmotorisk funktionsnedsättning förekom inte bland kontrollerna men var vanligt i diagnosgruppen (43%). Det fanns en signifikant korrelation mellan oralmotorisk funktionsnedsättning och vissa strukturella avvikelser och vissa aspekter på oral hälsa.

Jämfört med friska kontroller har personer med sällsynta diagnoser ofta svårt att medverka till tandbehandling och tandborstning. Dysmorfologi och oralmotorisk dysfunktion är vanligt förekommande i den här patientgruppen. Förstärkt profylax och tillgång till högspecialiserad tandvård är ofta motiverat.

Introduction

The concept of rare diseases

The Swedish National Board of Health and Welfare has defined the concept of rare diseases as: "A disease or disorder is defined as rare when it affects no more than 100 individuals per million population and leads to a marked degree of disability." (fewer than 1 in 10,000) (61). However, the concept of rare diseases varies between countries. The European Commission on Public Health defines rare diseases as "Life-threatening or chronically debilitating diseases which are of such low prevalence that special combined efforts are needed to address them" (39). A disease is defined as rare in most European countries when it affects fewer than 5 in 10,000 (10). In the USA, the definition is fewer than 7 in 10,000 (34) and, in Japan, 4 in 10,000 (22). Due to the pattern of inheritance, a disease could be rare in one population and common in another.

Most rare diseases (80%) have a genetic cause (10), but the aetiology could also be infections or embryopathy. In some diagnoses, the signs and symptoms are obvious from birth, while in others they appear later in childhood or in adult life and there are rare diseases that have a progressive course. Most often the disease is lifelong and, with only few exceptions, there is as yet no cure. Treatment generally focuses on relieving symptoms.

Oral health and oromotor function

If the nervous or musculoskeletal system is affected, there is an increased risk of oromotor dysfunction, such as difficulty with feeding, speech, facial expression, chewing and swallowing and saliva control (1, 32, 44, 45, 55). Impaired oromotor function and strength could also have a negative effect on facial growth (14, 15, 23, 24), oral clearance and jaw function (7, 34). Oro-cranio-facial anomalies including clefts, choanal atresia, craniofacial dysostosis, malar hypoplasia, micrognathia, hemifacial microsomia and tongue malformations are characteristic features in some congenital diseases. Congenital malformations affecting the orofacial area, as well as severe malocclusion, could also be expected to interfere with orofacial functions and with facial appearance (2, 3, 5, 62, 63).

Developmental delay, cognitive impairment and neuropsychiatric disorders are common in rare diseases affecting the brain. It has been shown in earlier studies that cognitive impairment is a risk factor for oral health, malocclusion and oromotor dysfunction (36, 37) and that behavioural problems have an impact on the ability to co-operate in oral hygiene procedures and dental care (16, 17, 27, 29, 39, 40, 41).

The database

In 1996, the Mun-H-Center (MHC), a Swedish national orofacial resource centre for rare diseases, created a database in order to collect data on oral health and orofacial function in rare diseases. Selected reports from the database are published on the internet (31). Some of the data in the database have been collected in connection with specific research projects and the results have been presented in scientific publications (8, 9, 25, 53, 54, 56, 57, 58, 59).

The aim

The aim of the present study was to provide an overview of general disabilities, oral health and oromotor function in individuals with rare diseases based on findings registered in the MHC database. To the best of our knowledge, the estimated prevalence of odontological manifestations and oromotor impairment in rare diseases as a group has not been published before.

The main research questions were: How do individuals with rare diseases cope with dental treatment and manage tooth brushing compared with healthy controls? How do intellectual disability and behavioural problems affect oral health? To what extent do individuals with rare diseases have odontological symptoms that demand specialised dental care and to what extent do they receive this service? How is oromotor function affected in individuals with rare diseases and how is this related to orofacial morphology and oral health?

Material and methods

The study group

The study group consisted of 1,829 individuals representing 169 different rare diseases and for the purpose of the present study it was divided into four subgroups following the ICD-10 classification system (Table 1). Group 1 consisted of "Mental and behavioural disorders" and "Congenital malformations, deformations and chromosomal abnormalities". Group 2 contained "Diseases of the nervous system" and Group 3 "Endocrine, nutritional and metabolic diseases". Group 4 contained diseases that affect single body systems: "Diseases of the blood and blood-forming organs and certain disorders involving the immune mechanism", "Diseases of the eye and adnexa", "Diseases of the ear and mastoid process", "Diseases of the circulatory system",

Table 1. Study population and controls.
Obs = MHC orofacial observation chart, Que = MHC questionnaire. Group 1: mental and behavioural disorders and congenital malformations, deformations and chromosomal abnormalities; Group 2: diseases of the nervous system; Group 3: endocrine, nutritional and metabolic diseases; Group 4: diseases that affect single body systems.

Subgroup	Number		Males		Females		3-6 years		7-12 years		13-19 years		> 19 years	
	Obs	Que	Obs	Que	Obs	Que	Obs	Que	Obs	Que	Obs	Que	Obs	Que
Group 1	1131	1177	565	576	566	601	436	450	415	428	202	196	78	103
Group 2	244	255	154	161	90	94	59	68	72	80	84	85	29	22
Group 3	194	232	101	127	93	105	68	83	77	86	34	42	15	21
Group 4	45	39	26	24	19	15	20	17	16	15	9	7	0	0
Total	1614	1703	846	966	768	869	583	618	580	609	329	330	122	146
Controls	137	132	82	78	55	54	46	53	77	73	14	6	0	0

Table 2. Diagnoses
The five most frequent rare diseases in each subgroup included in the study.
Obs = the number of individuals examined according to the MHC Observation chart.
Que = the number of individuals who filled out the MHC Questionnaire.

Subgroup	Diagnosis	Obs	Que
Group 1	22q11 deletion syndrome	107	80
	Angelman syndrome	64	71
	Rett syndrome	59	68
	Ehlers-Danlos syndrome	64	67
	Fragile-X syndrome	63	64
Group 2	Myotonic dystrophy type 1	73	79
	Duchenne muscular dystrophy	42	37
	Spinal muscular atrophy	28	38
	Becker muscular dystrophy	11	11
	Hereditary spastic paraplegia	10	10
Group 3	Williams syndrome	58	82
	Congenital adrenal hypoplasia	17	18
	Phenylketonuria	14	14
	Salla disease	12	12
	Carbohydrate deficient glycoprotein syndrome	11	10
Group 4	Nefrotic syndrome	10	11
	Langerhans cell histiocytosis	5	5
	Primary immunodeficiency	5	3
	Kostmann syndrome	3	4
	Kearns-Sayre syndrome	2	2

"Diseases of the digestive system", "Diseases of the musculoskeletal system and connective tissue" and "Diseases of the genitourinary system".

The mean age of the study group was 10.28 years (SD=7.627) and the median age was nine years (range 3-67 years). Rare diseases were selected according to the definition given by the Swedish National Board of Health and Welfare (a disease affecting fewer than 1 in 10,000 people).

Approximately 80% of the participants were recruited from the whole of Sweden through the family programme at the Ågrenska National Competence

Centre for Rare Diseases, Gothenburg, Sweden. The rest were regular patients referred to the MHC or other dental clinics in the Västra Götaland Region or participating in research projects.

The MHC questionnaire (30) was answered by 1,703 individuals, 1,614 were examined according to the MHC observation chart (30) and 1,488 did both. If an individual had more than one questionnaire or observation chart, data from the latest assessment were chosen for the present study. The five most frequent diagnoses in each subgroup are listed in Table 2.

The control group

In order to collect reference data from a group of healthy children, 220 children, aged 3-14 years (approximately 10% of the background population) were randomly selected from a Swedish community (Lindome). The MHC questionnaire was sent to the parents, with a letter explaining the purpose of the study and a request for their participation. If possible, the families that did not answer the letter were reminded by a telephone call two months later. The parents of 145 children (64%) accepted the invitation and gave signed consent that allowed a dentist and a speech-language pathologist to examine the child at home, at a day-care centre or at school. Four of these children were subsequently excluded from the study because they had a medical diagnosis. Seventy-nine children were not reached, of whom two declined participation and three were absent from school on the day of the examination. Finally, the control group consisted of 141 healthy children with a mean age of 8.11 years (SD=3.021) and a median age of 8 years (Table 1). Of those, 137 participated in a clinical examination, 132 answered a questionnaire and 130 did both.

Assessment

The clinical assessments of the study group were mostly made by members of a multi-professional team at the MHC, consisting of a hospital dentist, orthodontist, pedodontist, prosthodontist, stomatognathic physiologist and speech-language pathologist. The examiners were calibrated through regular meetings at which clinical cases, clinical photographs and dental casts were discussed and evaluated for a consensus agreement. Examiner agreement was tested on continuous data on 16 regular patients. The interexaminar variability relating to the variable "DFT" had an intraclass correlation (ICC) of 0.95 (CI: 0.85–0.99), the variable "maximum jaw opening (mm)" 0.94 (CI: 0.82-0.98) and the variable "horizontal overbite (mm)" 0.80 (CI: 0.32-0.94). A written manual with consensus-based definitions of the variables included in the examination protocol was used. The clinical examination of the control group was performed by the same dentist and speech-language pathologist.

The data were collected using the MHC questionnaire (30) and the MHC orofacial observation chart (30) and registered in the MHC database. The database was originally written in Microsoft Access and subsequently implemented in Microsoft SQL Server 2000.

Most patients were examined in a dental chair, but some sat on an ordinary chair, in a wheelchair, or on the floor. The only dental equipment used was a torch, a mirror and occasionally a probe. No X-rays were taken, with the exception of the regular patients referred to the clinic. Orofacial morphology, dental occlusion, oromotor function, oral health and oral hygiene were observed and evaluated by a dentist and a speech-language pathologist. Most evaluations were dichotomised and only pronounced deviations were noted. A three-point scale was used to assess oral hygiene: good, acceptable or poor. The ability to co-operate in the examination was presented on a three-point scale: "No or minor problems" (Patient is totally relaxed. Treatment is possible without problems. /Patient is relaxed but some reaction can be observed), "Moderate problems" (Patient is fairly relaxed. Treatment can continue if adapted to patient's reactions. /Patient is not relaxed. Reactions are considerable and treatment is obviously affected) and "Severe problems" (Patient consents to beginning treatment, but reactions are so severe that treatment is practically impossible to complete. /Patient refuses treatment.). The patient's ability to cope with dental treatment was also estimated on a visual analogue scale where the endpoints were "No problems" (0) and "Severe problems" (100). The number of primary and permanent teeth were counted and the number of decayed and filled teeth was checked with an ocular inspection (DFT/dft). Enamel caries was included in the caries-free group. The muscle tone and mobility of the facial, tongue and jaw muscles were evaluated by observation and palpation of the muscles and, if the participants were able to co-operate with this, by asking them to imitate lip, tongue and jaw movements.

Questions about medication, general disabilities, oral hygiene and dental care were answered by the parents, patients or assistants. The patients' ability

to brush their teeth was evaluated with marks on a visual analogue scale (VAS). Other questions were answered with "Yes" or "No". If a question could not be answered for any reason, it was left blank.

Ethical considerations

The study was approved by the ethics committee at the Sahlgrenska Academy, University of Gothenburg, and The Swedish Data Inspection Board.

Statistics

Statistical analyses were made using SPSS (version 21.0). Cross-tabulations were used for correlation analyses and group comparisons of categorical data ($\chi 2$ test). ANOVA and a t-test were used to analyse differences between groups of continuous variables. A logistic regression analysis was made in order to analyse risk factors for caries lesions. Descriptive statistics were shown as the mean and standard deviation (SD). The level of significance was set at $p<0.05$.

Results

General disabilities

The relative frequency of impaired functions reported in the questionnaire is presented in Figure 1. In Group 1, 21.3% of the participants reported multiple disabilities (a combination of four or more impaired functions), 20.2% in Group 2, 16.6% in Group 3 and 13.1% in Group 4.

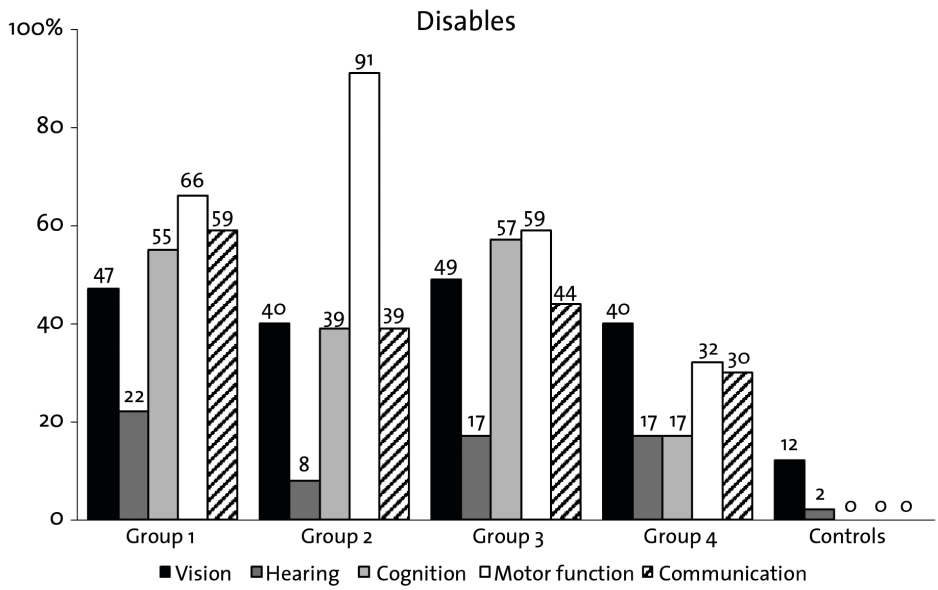
Coping with examinations and dental treatment

The results for the examiners' ratings of how calm and treatable the patients were at the time of the examination revealed that, in the study group, 60% had no or only minor problems, 29% had moderate problems and 11% had severe problems, while, in the control group, all the participants had no or only minor problems ($p<0.001$). There was no difference between groups within the study group ($\chi 2=5.078$, $p=0.534$) when it came to the ability to cope with examinations.

When the examiners overviewed and estimated the patient's ability to cope with dental treatment in general on a VAS, the mean VAS value for the study group was 33.21 (SD=35.0; CI=31.09-35.32) and the median was 17.0 (range=0-100). There were similar results in all subgroups except for the control group, which had a significantly lower result ($p<0.001$). In this group, the mean VAS value was 1.69 (SD=6.79; CI=0.54-2.83) and the median 0.0 (range=0-57).

The patients or parents were also asked to evaluate the patients' ability to cope with dental treatment

◎ Figure 1. Impaired functions reported
The relative frequency of impaired functions reported in a questionnaire by 1,703 individuals with rare diseases and 132 controls. Included subgroups: Group 1: mental and behavioural disorders and congenital malformations, deformations and chromosomal abnormalities; Group 2: diseases of the nervous system; Group 3: endocrine, nutritional and metabolic diseases; Group 4: diseases that affect single body systems.



and the corresponding results for the study group were a mean VAS value of 32.4 (SD=32.3; CI=30.8-34.0), a median of 20 (range=0-100) and, for the control group, a mean of 6.1 (SD=12.7; CI=3.9-8.3) and a median of 1.0 (range=0-70). If there were coping difficulties, they estimated the extent to which the difficulty coping with dental treatment was caused by fear or by disability. The mean VAS value for "fear" as the cause was 36.2 (SD=36.2; CI = 34.0-38.4) and the median was 20 (range=0-100). The mean for "disability" as a cause was 52.6 (SD=38.4; CI=50.3-55.0) and the median was 60 (range=0-100).

The majority of the individuals in the study group (53.1%) reported that they used the public dental service, 42.2% specialised dental care, 3.4% private dental care and 1.3% that they had no dental care. There was no significant difference between the four subgroups within the study group. All the controls but one, who had no dental care, used the public dental service. In the study group, 86.1% (84.9-89.2 %) stated that they received sufficient dental care and the corresponding figure in the control group was 97.7% ($\chi 2$=14.624, p<0.001). Of those that expressed dissatisfaction with dental treatment, the most frequently reported causes were requests for more frequent dental visits (41%), the dental teams' lack of knowledge of the diagnosis (14%) and need for specialised dental care (14%).

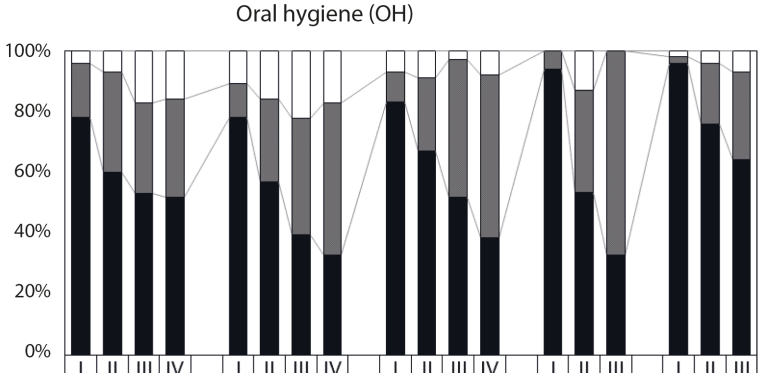
Oral health

When the participants were asked how they managed tooth brushing, the resulting mean VAS values for each subgroup were 51.0 (SD=37.0; CI=48.9-53.2) (Group 1), 49.7 (SD=37.7; CI=45.0-54.5) (Group 2), 54.3 (SD=36.0; CI=49.6-59.1) (Group 3), 38.8 (SD=31.6; CI=28.3-49.3) (Group 4) and 17.4 (SD=22.8; CI=13.4-21.4) (Controls). The range was 0-100 (median=49) for the study group and 0-93 (median=5.5) for the control group. They were also asked how tooth brushing went if they needed help and the mean VAS values were 32.9 (SD=32.4; CI=30.8-35.1) (Group 1), 20.4 (SD=25.1; CI=16.7-24.0) (Group 2), 28.5 (SD=29.5; CI=24.3-32.8) (Group 3), 20.0 (SD=29.3; CI=8.8-31.2) (Group 4) and 7.8 (SD=15.1; CI=3.9-11.6) (Controls). The range was 0-100 (median=17) for the study group and 0-80 (median=3) for the controls. The ability to manage tooth brushing had a negative correlation with age (r=-0.088, p<0.001), but this did not apply to the ability to manage tooth brushing when help was needed (r=0.009, p=0.752).

In a sub group of individuals 3-14 years of age, 67.1% in Group 1 had good oral hygiene, 61.8% in Group 2, 72% in Group 3 and 72.2% in Group 4, compared with 81.3% in the control group. The relative frequency of good oral hygiene was higher in the younger age groups in all subgroups (Figure 2).

The relative frequency of gingivitis was 32.6% in the study group and 20.4% in the control group (Table 3). There was no difference between subgroups when making comparisons within each age group, with the exception of the youngest children where the controls had less gingivitis compared with the study group (r=-0.111, p=0.042). There was a significant correlation between gingivitis and age (r=0.320, p<0.001).

In the youngest age group (3-6 years) the prevalence of caries-free individuals in Group 1 were



	Group 1				Group 2				Group 3				Group 4			Controls		
	I	II	III	IV	I	II	III	IV	I	II	III	IV	I	II	III	I	II	III
□ Poor OH	4	7	17	16	11	16	22	17	7	9	3	8	0	13	0	2	4	7
■ Acceptable OH	18	33	30	32	11	27	38	50	10	24	45	64	6	33	67	2	20	29
■ Good OH	78	60	53	32	79	57	39	33	84	67	52	39	94	53	33	96	76	64

Figure 2 – Oral hygiene (OH)
Oral hygiene evaluated in four subgroups of rare diseases (N = 1,614) and in a group of healthy controls (N = 137). Included age groups: I = 3-6 years, II = 7-12 years, III = 13-19 years, IV >19 years.

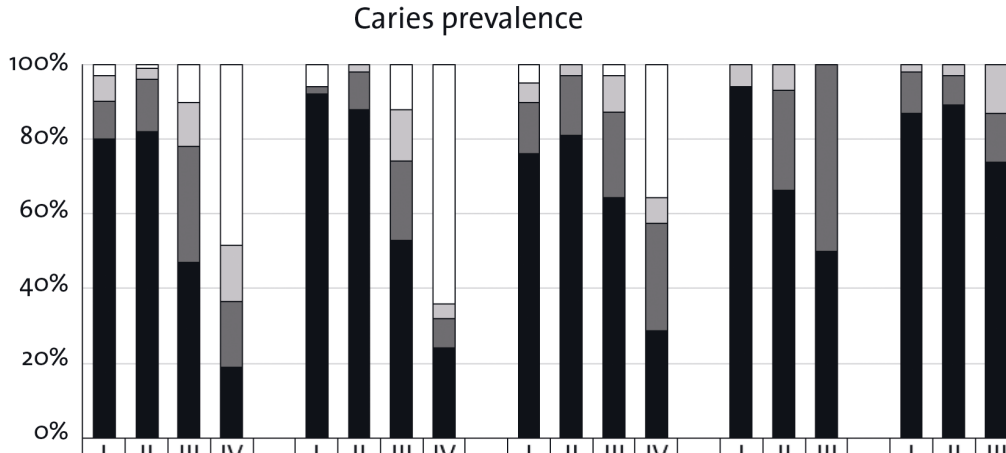
Table 3. Gingivitis
The frequency of gingivitis in four subgroups of rare diseases and a control group.

Subgroup	3–6 yrs		7–12 yrs		13–19 yrs		> 19 yrs		All ages	
	N	%	N	%	N	%	N	%	N	%
Group 1	72	18.3	147	37.9	94	49.2	45	59.2	358	34.2
Group 2	4	7.5	21	29.2	46	59.0	17	58.6	88	37.9
Group 3	8	12.7	16	22.2	17	50.0	9	60.0	50	27.2
Group 4	1	5.0	7	43.8	4	44.4	-	-	12	26.7
Total	88	15.3	213	34.1	164	50.2	71	59.2	536	32.6
Controls	3	6.5	22	28.9	3	20.0	-	-	28	20.4

81.1%, in Group 2, 92.0% in Group 3, 76.2% in Group 4, 94.1% and in the control group 87.0%. The relative frequency of individuals without caries in the permanent dentition in the 7-12 year age group was 82.2% in Group 1, 87.9% in Group 2, 81.4% in Group 3, 66.7% in Group 4 and 89.5% in the control group. In the 13-19 year age group, the results were 47.1% in Group 1, 54.7% in Group 2, 64.5% in Group 3, 50.0% in Group 4 and 73.3% in the control group (Figure 3). No significant difference was found between the study group and the controls ($\chi 2$=2.763, p=0.096). There was a significant association in the number of caries-free patients related to age within subgroups 1 ($\chi 2$=181.932, p<0.001), 2 ($\chi 2$=53.944, p<0.001) 3 ($\chi 2$=17.759, p<0.001) and 4 ($\chi 2$=6.539, p=0.038) but not in the control group ($\chi 2$=2.860, p=0.239).

A logistic regression analysis was made with number of caries lesions (0 vs ≥1) as the dependent factor and oral hygiene, coping with dental examination, oromotor dysfunction, intellectual disability and mobility dysfunction as covariates. The model

Figure 3 – Caries prevalence
Caries prevalence evaluated in four subgroups of rare diseases and in a group of healthy controls. Included age groups: I = 3-6 years, II = 7-12 years, III = 13-19 years, IV >19 years. In age group I only primary teeth were included and in the other age groups only permanent teeth.



	I	II	III	IV	I	II	III	IV	I	II	III	IV	I	II	III	I	II	III
	Group 1				Group 2				Group 3				Group 4			Controls		
□ DFT ≥ 7	3	1	10	49	6	0	12	64	5	0	3	36	0	0	0	0	0	0
▒ DFT = 4–6	7	3	12	15	0	2	14	4	5	3	10	7	6	7	0	2	3	13
▒ DFT = 1–3	10	14	31	18	2	10	21	8	14	16	23	29	0	27	50	11	8	13
■ DFT = 0	81	82	47	19	92	88	53	24	76	81	65	29	94	67	50	87	90	73

Table 4. Caries – possible risk factors
The results from a logistic regression analysis of possible risk factors for caries (no/yes) including coping with dental treatment (no, moderate or severe problems), oral hygiene (good, acceptable or poor), oromotor dysfunction (no/yes), intellectual disability (no/yes) and mobility dysfunction (no/yes). Nagelkerke r2=0.118.

Variable	B	p-value	OR	95% CI
Coping		0.055		
- no problem (ref)				
- moderate problem	-0.45	0.020	0.64	0.44-0.93
- severe problem	0.01	0.963	1.00	0.56-1.77
Oral hygiene				
- good (ref)				
- acceptable	1.04	0.000	2.82	2.03-3.91
- poor	1.60	0.000	4.97	2.97-8.3
Oromotor dysfunction				
- no				
- yes	-.21	0.269	0.863	0.56-1.17
Intellectual disability				
- no (ref)				
- yes	0.1	0.948	0.99	0.69-1.41
Mobility dysfunction				
- no (ref)				
- yes	-0.25	0.153	0.78	0.56-1.1

revealed that the variable oral hygiene was significantly associated with caries (Table 4). There was also a significant correlation between the frequency of caries and oral hygiene (r=0.227, p<0.001).

Two thirds of the individuals with severe tooth abrasion (82/121) were reported to grind their teeth. However, there was a vast variation between diagnoses. Severe tooth abrasion was most common in Aicardi syndrome (50%), CHARGE syndrome (31.3%), Rubinstein Taybi syndrome (29.4%) and Rett syndrome (28.6%) and was absent or almost absent in Duchenne muscular dystrophy (0%), Williams syndrome (0%), 22q11 deletion syndrome (2.1%) and myotonic dystrophy type 1 (4.2%). In the study group, 30.3% reported tooth grinding (18.2-34.1%), while the corresponding figure among the controls was 14.5%. There was a significant correlation between severe tooth abrasion and tooth grinding (r=0.265, p<0.001).

Orthodontics and jaw function

Children and adolescents aged 7-19 years were chosen for the presentation of craniofacial anomaly, malocclusion and other structural deformities in all subgroups (Table 5). Significant differences between the study group and the control group were found in craniofacial anomaly ($\chi 2$=10.192, p<0.001), postnormal bite ($\chi 2$=5.972, p<0.015), frontal open bite ($\chi 2$=21.680, p<0.001), frontal open bite with molar contact only ($\chi 2$=10.515, p=0.001), long face ($\chi 2$=6.580, p=0.01), narrow palate ($\chi 2$=19.904, p<0.001) high palate ($\chi 2$==29.086, p<0.001) and reduced mobility in TMJ ($\chi 2$=7.293, p<0.007).

In the 13-19 year age group, according to the questionnaire, 43.7% in Group 1 had received orthodon-

Table 5. – Oromotor impairment
The relative frequency of oromotor impairment in four subgroups of rare diseases (N = 1,614) and in a group of healthy controls (N = 137).

Subgroup	Oromotor impairment	
	Yes %	No %
Group 1	37.4	62.6
Group 2	51.5	48.5
Group 3	47.2	52.6
Group 4	11.1	88.9
Controls	0	100

◎ Table 6. Structural deformities
The frequency of craniofacial anomaly, malocclusion and other structural deformities in four subgroups of rare diseases and a control group. Age groups II-III (7-19 years). TMJ=Temporomandibular joint.

	Group 1		Group 2		Group 3		Group 4		Controls	
	N	%	N	%	N	%	N	%	N	%
Craniofacial anomaly	48	8.2	2	1.3	1	0.9	0	0	0	0
Neutral bite	335	57.7	101	67.3	61	58.1	16	58.1	62	64.0
Postnormal bite	157	27.4	28	18.9	27	25.7	5	20.0	24	26.4
Prenormal bite	77	13.4	18	12.2	16	15.1	4	16.0	5	5.5
Frontal open bite	108	19.0	44	29.7	21	19.8	2	8.0	3	3.3
Molar contact only	50	8.9	20	13.7	9	8.6	2	8.0	1	1.1
Deep bite/gingival contact	38	6.7	5	3.4	7	6.7	0	0	7	7.7
Long face	62	11.1	18	12.2	4	3.9	1	4.3	3	3.3
Narrow palate	139	25.0	29	19.9	18	18.0	1	4.2	4	4.4
High palate	129	22.3	28	18.9	17	16.2	2	8.0	0	0.0
Reduced mobility in TMJ	29	5.2	6	4.2	2	2.0	0	0.0	0	0.0

tic treatment, 48.7% in Group 2, 31.3% in Group 3, 14.3% in Group 4 and 33.3% in the Control group. At the same time, 53.1% in Group 1, 58.5% in Group 2, 34.8% in Group 3 and 57.1% in Group 4 reported having had/still having faulty dentition compared with none in the Control group ($\chi 2$=15.492, p=0.004).

Oromotor function

Oromotor impairment was a frequent finding in the study group (43.1%) and was absent among the controls. There were, however, significant differences between the various subgroups within the study group ($\chi 2$=51.302, p<0.001) and the relative frequency varied between 11.6% and 59.6% (Table 6). Oromotor impairment was equally distributed between males and females ($\chi 2$=0.128, p=0.721). The teenagers (49.5%) and the adults (55.2%) more frequently had impaired oromotor function than the children aged 3-6 years (43.1%) and 7-12 years (37.0%). In some diseases (represented by >20 participants), more than 75% of the individuals had oromotor impairment: Möbius sequence (100%), Rett syndrome (96.4%), Cri-du-chat syndrome (96.3%), Cornelia de Lange syndrome (93.8%), Angelman syndrome (93.5%), congenital/childhood myotonic dystrophy (88.9%) and Williams syndrome (75.9%).

There was a significant correlation between oromotor impairment and certain kinds of malocclusion, structural malformations and oral-health issues but not with craniofacial anomalies (Table 7).

The majority (69.3%) of the individuals with oromotor impairment had an open mouth at rest. An open mouth at rest was a frequent finding in the study group (42.1%) compared with the controls (5.1%). The relative frequency of patients with an open mouth at rest in the study group was 41.0% (Group 1), 46.4% (Group 2), 48.9% (Group 3) and 18.6% (Group 4) respectively.

Discussion

The results of this Swedish database study clearly show that impaired oral health and oromotor function are frequent findings in individuals with rare diseases and seldom exist in healthy children. In the present study, "oral health" was defined as: "Oral health is a state of being free from caries, gingivitis, periodontal disease, mucosal lesions, TMJ and occlusal dysfunction". Since oral health is found to be related to the socioeconomic situation of the individual as well as that of society, these results could not be generalised to all populations (35, 42). However, similar results were found in an American population with intellectual disability (43).

It was presupposed that the aetiology of a medical disease would have an impact on oral health and oromotor function and the rare diseases in the database were therefore clustered together in subgroups using the ICD-10 classification system. Group 1 was

Table 7. Oromotor function correlated with odontological findings The correlation between oromotor function and odontological symptoms such as malocclusion, craniofacial anomalies, structural malformations and oral-health aspects in individuals with rare medical conditions (N=1,614).

Odontological symptoms	Oromotor impairment		p	r
	Yes (%)	No (%)		
Craniofacial anomaly	8.1	6.1	0.139	0.038
Postnormal bite	26.5	17.6	0.000	0.107
Prenormal bite	17.8	13.0	0.012	0.067
Frontal open bite	34.6	17.1	0.000	0.202
Molar contact only	13.8	4.6	0.000	0.162
Deep bite – gingival contact	7.3	4.0	0.007	0.072
Long face	12.4	5.0	0.000	0.133
Narrow palate	25.3	15.4	0.000	0.123
High palate	23.9	15.1	0.000	0.111
Reduced mobility in TMJ	8.6	2.8	0.000	0.129
Severe tooth wear	16.9	5.3	0.000	0.190
Gingivitis	43.3	26.8	0.000	0.172
Supra gingival calculus	15.0	7.5	0.000	0.121

the largest subgroup and comprised mainly genetic syndromes; Group 2 primarily involved neuromuscular diseases, Group 3 endocrine and metabolic diseases and Group 4 diagnoses that affect a single body system. The present results in general showed that there were significant differences between the subgroups in many of the tested variables and the validity of the present subdivision was thereby confirmed.

Many individuals in the study group had moderate or severe difficulty coping with dental examinations and with dental treatment compared with none in the control group. Despite problems co-operating, the percentage of patients receiving dental care was the same in both the study group and the control group. The large number of patients with treatment difficulties was probably the main reason for receiving specialised dental care (26). According to the parents' reports, the treatment difficulties were more due to disability than to dental fear. In addition, many of these patients had difficulties related to tooth brushing.

Before puberty, the majority had good oral hygiene, but in the older age groups oral hygiene was not as good. This might be attributed to the process when the responsibility for oral hygiene is transferred from the parents to the young people. However, in Group 1, good oral hygiene was maintained for longer. One explanation for this could be that many of these individuals were unable to manage tooth brushing themselves and they were therefore dependent on help from parents or assistants. Gingivitis is linked to poor oral hygiene and has been found to be significantly correlated with age in previous studies (4, 47). These findings were confirmed in the present study, even though contradictory findings have been reported (21)

Almost all the patients in the 9-12 year study group, as well as in the control group, were caries free. The corresponding numbers in other populations have been reported to be significantly lower (36). However, when Swedish children in general are compared with children in several other European countries, the DMFT in 12-year-old children are comparable (4).

The high frequency of caries in the adult group could be explained by a less well developed dental care system when they were young. *Hugoson A et al.* (2008) (18) showed that the general oral health status improved over time in Swedish society from 1973 to

2003 (18, 19, 20). There has been a global trend for the number of DFT among 12 year olds to decrease during the last 15 years (4, 52).

A high percentage (40%) of the individuals in the study group had received orthodontic treatment. This should be compared with a general Swedish population where only 25% of teenagers receive orthodontic treatment (52). The present study clearly shows that patients with rare diseases as a group have a more extensive need for orthodontic treatment due to a higher frequency and increased severity of malocclusion compared with healthy controls. The results of the present study also support the idea that there is interplay between orofacial form and orofacial function. Although there was a significant correlation between different types of malocclusion and oromotor impairment, the underlying cause of this correlation cannot be concluded from these figures alone. In some diagnoses, the malocclusion could have a genetic cause and, in other cases, it could be the result of negative facial growth due to breathing pattern, oromotor function, oral habits or head position (11, 14, 15, 23, 24, 50). In those diagnoses where malocclusion is found to be secondary to any kind of dysfunction, oromotor assessment and treatment should be considered in the orthodontic treatment planning (6, 12, 47, 48, 50).

Diet is regarded as the main risk factor for caries development, but this was not investigated in this study (28, 49). Dental care on a regular basis has also been proven to be important for oral health, as it has been shown that the number of dental visits is negatively correlated to tooth morbidity in adults with mental disabilities (13). However, in the present study, it was found that poor oral hygiene was the main risk factor for caries development and that the main risk factor for poor oral hygiene was intellectual disability. These results point to an indirect relationship between caries development and intellectual disability. It is therefore important to emphasise that intellectual disability per se is not the cause of developing caries. A great deal of effort therefore needs to be concentrated on an effective prophylactic regimen for this group of patients.

Gingivitis, supragingival calculus and severe tooth abrasion were found to be strongly correlated to oromotor dysfunction in the present study group. Oromotor impairment and reduced oral sensibility have an impact on the ability to keep the oral cavity clean and could therefore affect oral health. As an example, *Engvall & Birkhed* (1997) showed that the time taken for oral sugar clearance was prolonged in individuals with myotonic dystrophy and oromotor impairment compared with healthy individuals (7).

Study limitations

Most participants were enrolled in the study in connection with family stays at the Ågrenska National Competence Centre for Rare Diseases. The extent to which the children from these families are representative of their diagnoses is not known. Of those that attended the family stays, almost all were included. The severity of symptoms, as well as the social situation of the family, could probably have an impact on the choice or opportunity to attend the family stays. However, they were not selected because of oral symptoms but because they had a rare medical condition or diagnosis. The registration of caries was not supported by X-ray examinations and the number of caries-free patients could therefore have been overestimated and, as there was no access to earlier dental records, the history of missing teeth could not be accounted for. The fact that there were different examiners could also have had an impact on the results. The examiners worked together in a team and were calibrated through regular meetings and clinical co-operation and the tested variables were well defined and, as a result, no systematic deviations related to examiner agreement were expected to occur.

Clinical implications

Individuals with rare diseases often require extra prophylactic dental care and access to specialised dental care in order to prevent oral diseases. They often have symptoms and deviations from multiple body systems and therefore require a multidisciplinary treatment plan, including different medical and dental disciplines. In order to offer treatment adapted to each individual, it is extremely important to involve parents, as well as the surrounding network, in the treatment planning. Most patients and parents found that the Swedish dental system provided sufficient care, but the knowledge relating to the special needs of patients with rare diseases could be improved.

Conclusions

– More than one third of the individuals with rare diseases have difficulty coping with dental treatment and tooth brushing.

– Oral hygiene was shown to be a significant predictor of individuals with caries, the worse oral hygiene the higher likelihood of having caries.

– Individuals with rare diseases often have odontological symptoms that require specialised dental care.

– Malocclusion and oral-health factors could be correlated to oromotor dysfunction in individuals with rare diseases.

– Oromotor dysfunction is common in rare diseases where more than one body system is affected.

Acknowledgements

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The influence of immigrant background on the choice of sedation method in paediatric dentistry

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Abstract

◎ The effects of immigration on the demographics of the Swedish population have changed the situation for many dental care providers, placing increased demand on cultural competence. The aim of this investigation was to study the choice of sedation method among children with immigrant background, referred to paediatric dentistry specialists, because of behaviour management problems or dental fear in combination with treatment needs.

The material consisted of dental records from children referred to two clinics for paediatric dentistry: 117 records from children with an immigrant background and 106 from children with a non-immigrant background. Information about choice of sedation method (conventional treatment, conscious sedation with midazolam, nitrous oxide, or general anaesthesia) and dental status was collected from the records. The number of missed appointments (defaults) was also registered. Binary logistic regression analyses were used to calculate the influence of potential predictors on choice of sedation method.

The mean age of the patients in the immigrant group was 4.9 yrs, making them significantly younger than the patients in the non-immigrant group (mean 5.7 yrs). In the immigrant group, 26% of the patients defaulted from treatments, while the corresponding frequency was significantly lower for the reference group (7%). The numbers of primary teeth with caries and permanent teeth with caries were positively and significantly correlated with the choice of treatment under general anaesthesia. Conscious sedation was used significantly more often in younger children and in the non-immigrant group, while nitrous oxide was preferred in the older children.

In conclusion, conscious sedation was more frequently used in the non-immigrant group. The choice of sedation was influenced by caries frequency and the age of the child.

Key words

Conscious sedation, general anaesthesia, immigrant children, nitrous oxide, paediatric dentistry.
Running title: Sedation methods in paediatric clinics

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Faktorer av betydelse för val av sederingsmetod vid pedodontikliniker

ANDREAS DAHLANDER, LEIF JANSSON, KERSTIN CARLSTEDT, MARGARET GRINDEFJORD

Sammanfattning

◎ Det svenska samhället har blivit mera mångkulturellt till följd av ökad invandring. Detta ställer ökade krav på vårdgivare vad avser patientbemötande och kulturell kompetens.
Syftet med denna studie var att undersöka vilka faktorer som styrde valet av sederingsmetod bland barn med och utan invandrarbakgrund som remitterats till specialistklinik i pedodonti på grund av behandlingssvårigheter eller tandvårdsrädsla.

Materialet bestod av patientjournaler från barn som remitterats till två specialistkliniker i pedodonti. Studiegruppen utgjordes av 117 journaler från barn med invandrarbakgrund samt en kontrollgrupp bestående av 106 journaler från barn med icke invandrarbakgrund. Uppgifter om val av sederingsmetod (konventionell behandling, lustgas, bensodiazepiner (Midazolam) eller generell narkos) och odontologiskt status samlades ur journalerna. Antalet uteblivanden från bokade besök registrerades likaså. Binär logistisk regressionsanalys användes för att analysera inverkan av potentiella prediktorer på valet av sederingsmetod.

Medelåldern hos patienterna med invandrarbakgrund var 4.9 år. Dessa individer var betydligt yngre än patienterna i icke invandrargruppen (medelålder 5.7 år). I invandrargruppen uteblev 26 % av patienterna från bokade besök och motsvarande frekvens bland patienter utan invandrarbakgrund var signifikant lägre (7 %). Behandling under narkos utfördes signifikant oftare hos individer med stort antal karierade primära och permanenta tänder. Sedering med bensodiazepiner var signifikant vanligare bland yngre barn i icke-invandrargruppen. Lustgas användes i större utsträckning bland äldre barn.

Sammanfattningsvis var sedering med besodiazepiner vanligare bland barn med icke-invandrarbackgrund. Kariesfrekvens och patientens ålder var faktorer som hade en signifikant betydelse för valet av sederingsmetod.

Introduction

Increased immigration has led to increased cultural and ethnic diversity in Swedish society, with nearly 18 per cent of the population having an immigrant background (16). In the County of Stockholm, the percentage of the population with immigrant background ranges from 11 per cent in the northwest (Ekerö) to 41 per cent in the south (Södertälje) (16). This demographic alteration has changed the situation for many dental care providers, placing increased demand on cultural competence.

Dental fear (DF) and dental behaviour management problems (DBMP) are well-known difficulties in paediatric dentistry. They both have a complex aetiology. DBMP may be defined as uncooperative and disruptive behaviour resulting in delay of treatment or rendering treatment impossible. DF is based on the experience of fear in relation to a threatening stimulus connected with dental treatment (9,13). The cause of DF is considered to be multifactorial. Among other factors, psychological aspects, temperament, and parental dental fear are decisive for development of DBMP and DF (10). Research on DBMP and DF in immigrant children is limited, and associations between immigrant background and DBMP or DF have not yet been described.

Dental treatment is challenging for young children. A study from Norway revealed that children who had caries experience at five years of age were more fearful at ten years of age compared with a group of children with no caries (14). A relationship between socioeconomic status and dental fear has been reported (1,6). It has also been found that children and adolescents referred because of DBMP often have a burdensome life and family situation. Children receiving support from school psychologists or other psychosocial professionals are overrepresented among children referred because of DBMP (1).

Immigrant children and children from families with low socioeconomic status have a higher risk of developing caries, because of interaction with risk factors such as frequent use of sweet drinks and irregular tooth-brushing (6,15). Consequently, these children often have a more extensive need for dental treatment compared to other children (2,11,17). Early and negative experiences, such as multiple extractions and previous experience of toothache, may contribute to DBMP and DF (7).

Strategies used in dentistry for managing anxiety and behaviour problems can be divided into two main categories: pharmacological and non-pharmacological (8,12). Behavioural techniques which do not involve drugs, such as the tell-show-do method, play an important part (12). However, some children still do not tolerate dental treatment, and sedation with nitrous oxide or conscious sedation with benzodiazepines may be considered. In severe cases, general anaesthesia is required (8). For a successful outcome when treating anxious and uncooperative paediatric patients, a suitable choice of treatment modality is crucial.

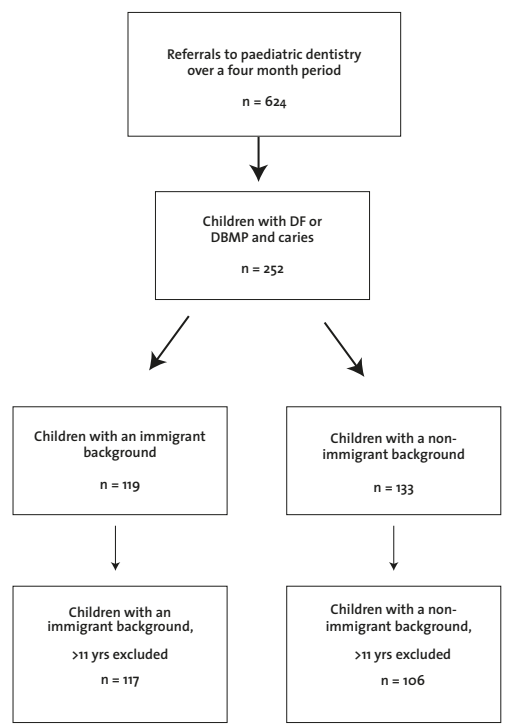
The aim of this investigation was to study the choice of sedation method among children with immigrant background referred to specialists in paediatric dentistry because of DBMP or DF in combination with dental treatment needs in relation to age and gender

Material and Methods

Participants and procedure

The material consisted of dental records from all children referred to the clinic for paediatric dentistry at the Eastman Institute in Stockholm and the clinic for paediatric dentistry in Sollentuna during a period of four months in 2005 and 2006. The total number of referrals was 624, and 252 of these con-

Figure 1. Flow chart illustrating the selection procedure.



cerned DBMP or DF in combination with caries. In this group, 119 children were considered to have an immigrant background and 133 to have a non-immigrant background (Figure 1).

The definition of immigrant background in this context is having been either born abroad, or born in Sweden with at least one parent born abroad.

The children with a non-immigrant background were older than the children with an immigrant background, and so children older than 11 were excluded in order to make the two groups more equal with respect to age. The final study group consisted of 117 dental records from children with an immigrant background (the immigrant group) and 106 dental records from children with a non-immigrant background (the reference group). These records were analysed with regard to variables concerning the patients' dental status and the treatment provided at the paediatric dentistry clinic.

Information about choice of sedation method (conventional treatment, conscious sedation with midazolam, nitrous oxide-oxygen, or general anaesthesia) and dental status was collected from the records, where dental caries had been diagnosed by clinical examination and with the aid of radiographs. The number of missed appointments (defaults) was also registered.

Ethical considerations

All data were treated confidentially, and approval from the research ethics committee at

Karolinska Institutet was obtained prior to data collection (ref: 2007/1128-31/3).

Statistics

All statistical calculations were performed with a statistical software package (IBM SPSS Statistics 19.0). A chi-square test was used to study the associations between categorical variables. One-way variance analysis was adopted to analyse differences between groups according to number of teeth with caries. Comparisons between groups according to caries frequency were performed using multiple linear regression analyses. Binary logistic regression analyses were used to calculate the influence of potential predictors on choice of sedation method. Results were considered statistically significant at $p<0.05$.

Results

The frequency distribution according to study group, age, and gender is presented in Table 1.

The mean age of the patients in the immigrant group was 4.9 yrs (S.D. 2.0), making them significantly younger than the patients in the reference group (mean 5.7 yrs, S.D. 2.4; $p=0.01$).

The choice of sedation method differed significantly between treatment groups ($p<0.01$, Table 2). The most frequent method in both groups was conscious sedation with midazolam. Conventional therapy was used in 33% of the cases in the immigrant group, and 12% in the non-immigrant group (Table 2). This difference was statistically significant ($p<0.001$).

The frequency distributions of caries in primary and permanent teeth for the immigrant group and the non-immigrant group are illustrated in Figures 2 and 3, respectively. The mean number of primary teeth with caries was 5.1 (S.D. 4.8) in the immigrant group and 3.9 (S.D. 3.0) in the non-immigrant group. This difference was not significant after compensation for age and gender. However, the mean number of permanent teeth with caries was significantly higher ($p=0.01$) for children in the non-immigrant group (mean 0.96, S.D. 1.9) compared to children with an immigrant background (mean 0.28, S.D. 0.95) when age and gender were included as independent variables in multiple regression analyses.

The choice of sedation method differed significantly depending on number of primary and permanent teeth with caries ($p<0.001$, Table 3). General anaesthesia was frequently used for patients with a high number of caries lesions. Those who were treated conventionally had a lower caries frequency (Table 3).

In the immigrant group, 26% of the patients de-

◉ **Table 1.** Frequency distribution according to treatment group, age, and gender.

Age (yrs)	Immigrant group		Non-immigrant	
	Females	Males	Females	Males
1-5	42	42	29	24
6-11	16	17	21	32
Total	58	59	50	56

Table 2. Frequency distributions (%) according to choice of sedation method in the immigrant group and the reference group.

Sedation method	Immigrant group	Non-immigrant group
Conventional treatment	33	12
Nitrous oxide	20	30
Conscious sedation	37	45
General anaesthesia	10	13

Table 3. Mean (S.D.) number of teeth with caries per patient, stratified according to sedation method.

Sedation method	Number of primary teeth with caries, mean (S.D.)	Number of permanent teeth with caries, mean (S.D.)
Conventional treatment	3.2 (3.8)	0.38 (1.1)
Nitrous oxide	3.8 (3.2)	0.94 (1.4)
Conscious sedation	4.7 (4.0)	0.21 (0.71)
General anaesthesia	7.9 (4.9)	1.8 (3.2)

Figure 2. Distribution of decayed primary teeth in each study group.

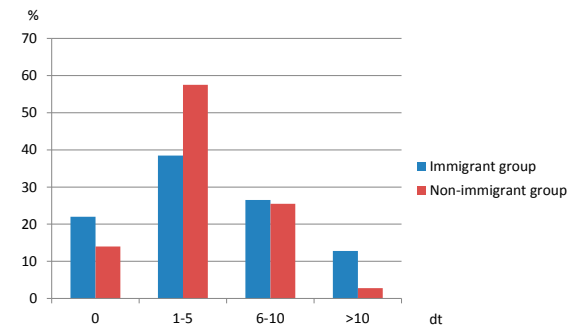
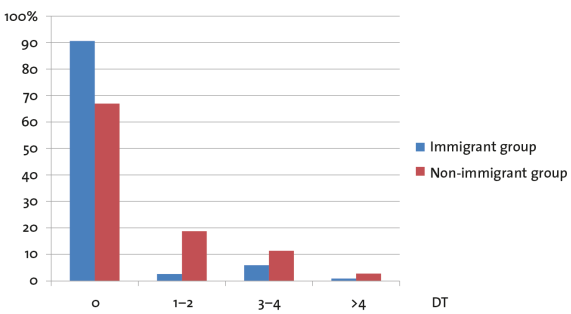


Figure 3. Distribution of decayed permanent teeth in each study group.



faulted from treatments, while the corresponding frequency was significantly lower for the non-immigrant group (7%, p=0.01).

The logistic regression analyses showed that the number of primary teeth with caries and the number of permanent teeth with caries were positively and significantly correlated with the decision to choose treatment under general anaesthesia (p<0.01, Table 4). Conscious sedation was used significantly more often in younger children (p<0.01) and in the non-immigrant group (p=0.04), while nitrous oxide was preferred in the older children (p<0.01, Table 4).

Discussion

This study focused on the choice of sedation method among children with and without an immigrant background. Before exclusion of children older than 11 years of age, the mean age was lower in the immigrant group than in the reference group. This could be explained by the fact that immigrant children develop caries earlier than non-immigrant children (3), and are thus referred to a specialist at a lower age. This in turn is partially due to early-established habits that promote the development of caries, such as high and frequent consumption of caries-risk products (2, 15).

The retrospective design of the study limits our capacity for a proper selection of variables. In addition, the participants were not randomly selected in either study group; their inclusion depended on the tendency in general dentistry to refer the patients, which may have differed between the two groups.

High frequencies of children with immigrant background, nearly half of the patients, were referred to the two clinics because of caries and DBMP. This result could be expected, since DBMP and DF are more frequent among children with caries. There was no significant difference in number of decayed primary teeth between the immigrant group and the reference group. The explanation for this could be that all patients were referred because of caries. Otherwise, one could expect to find more caries in the immigrant group than the reference group, since previous cohort studies have shown a higher prevalence of caries among immigrant children (6, 15). The number of decayed permanent teeth was higher in the reference group than in the immigrant group, which was unexpected. However, this may have been due to a higher tendency among general dentists to refer children with an immigrant background.

The choice of treatment differed significantly between groups. Conscious sedation was the most fre-

◎ Table 4. The results of stepwise logistic regression analyses with sedation methods as dependent variables and conventional treatment was used as reference treatment. Independent variables were included in the model if p<0.05.

Dependent variable	Independent variables	Odds ratio	Confidence interval	P
General anaesthesia	Number of primary teeth with caries	1.5	(1.1; 2.0)	<0.01
	Number of permanent teeth with caries	1.8	(1.3; 2.6)	<0.01
Conscious sedation	Age	0.7	(0.62; 0.88)	<0.01
	Immigrant group	0.5	(0.30; 0.92)	0.04
Nitrous oxide	Age	1.3	(1.1; 1.5)	<0.01

quently used method in both groups, which can be explained by the low age in the referred material. Sedation with benzodiazepines is a preferred method in younger children, since sedation with nitrous oxide needs more cooperation from the patient and so is more suitable for older and more mature children (5). Conventional treatment was more frequently used in the immigrant group compared to the non-immigrant group. There is no obvious explanation for this. One reason could be that dentists are less inclined to use sedation among immigrant children. Another hypothesis might be that general dentists are less likely to use the tell-show-do method with immigrant children; if this method was not tried in general dentistry before referral to the paediatric clinic, this might increase the probability of its being adopted in the specialist clinic. In addition, if there is a higher predisposition among general dentists to refer children with an immigrant background, then the non-immigrant children who are referred might constitute a group of more complicated cases, and conscious sedation might be used more often to manage treatment in more severe cases. Another reason could be that the parents of immigrant children have a negative attitude towards sedation.

However, in the final analyses, the significant factor influencing the choice of sedation with general anaesthesia was the number of decayed teeth. Consequently, patients with few decayed teeth were often treated conventionally, while their counterparts with a high frequency of decayed teeth underwent heavier sedations and even general anaesthesia in the most severe cases. Finally, after compensating for caries frequency and immigrant background, conscious sedation was more frequently used in the youngest group and nitrous oxide was more common among older children.

This study confirms that a large proportion of child patients referred to specialists in paediatric dentistry are children with an immigrant background. Our results indicate an extensive requirement for dental care among these patients. There is also a suggestion that there could be disparities in the choice of sedation method between children with an immigrant background and those without such a background. However, these differences could be explained by factors such as caries frequencies and the age of the patients. We also found that immigrant children had a higher frequency of missed appointments in comparison to the non-immigrant group. This could be regarded as avoidance of treatment and a sign of dental fear and anxiety, but it could also be an expression of lack of trust in the dental health system or a low priority assigned to dental care (4, 18). There is a need for further studies of child dental patients with an immigrant background, to gain knowledge about this group and hence reduce the disparities between children with and without an immigrant background.

In conclusion, conscious sedation was more frequently used in the non-immigrant group. The choice of sedation was also influenced by caries frequency and the age of the child. Children with immigrant background were referred at a lower age than children with non-immigrant background. .

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Dental caries and associated factors in a group of Swedish snus users

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◎ Abstract

The aim of this study was to investigate the effects of the Swedish moist powder tobacco product known as "snus" on dental caries and to measure the pH fall in dental plaque. The subjects comprised male and female adults between 26 and 62 years of age (n=102), all habitual snus users for ≥ 10 years. The control group (n=101) consisted of similar individuals in terms of gender, age and educational level but with no tobacco use for ≥ 10 years. A clinical and radiographic examination and a questionnaire were completed. The pH fall after a sucrose rinse was estimated *in situ* in 10 randomly selected subjects per group. The salivary secretion rate was higher in snus users than non-users (2.5 vs 2.2 ml/min, p=0.005). There was no statistically significant difference regarding salivary buffer capacity. No differences were found between the two groups in terms of the plaque index, primary or secondary enamel and dentine caries, DFS and salivary counts of mutans streptococci or lactobacilli. The pH fall was somewhat more pronounced among non-users compared with snus users (NS). Snus users had a lower intake of snacks between meals and a less frequent intake of cookies (p=0.000). Furthermore, snus users had a mean gingival index (±SD) for the whole dentition of 20.4 ± 18.2, while the index for non-users was 14.4 ± 13.9 (p=0.009); the corresponding values for teeth 13-23 were 14.9 ± 20.6 and 7.7 ± 11.9 respectively (p=0.003). To conclude, this clinical study revealed no statistically significant differences in caries prevalence between snus users and non-users and only minor differences regarding different cariesassociated factors.

Key words

Dental caries, plaque pH, snuff, snus

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Karies och kariesrelaterade faktorer hos en grupp svenska snusare

Lena Hellqvist, Margot Rolandsson, Anders Hugoson,
Peter Lingström, Dowen Birkhed

Sammanfattning

◉ Syftet med föreliggande undersökning var att studera effekten av snus på karies och på pH-förändringar i dentalt plack. Försökspersonerna bestod av 102 vuxna individer, män och kvinnor, i åldrarna 26 till 62 år som alla snusat dagligen i ≥ 10 år. Som kontrollgrupp matchades 101 individer mot försökspersonerna avseende ålder, kön och utbildningsnivå, men som inte använt tobak de senaste 10 åren. Samtliga individer besvarade ett frågeformulär och genomgick en klinisk och radiologisk undersökning. Förändringar i pH i plack efter sköljning med sackaros bestämdes *in situ* hos ett slumpmässigt urval av 10 individer i vardera gruppen. Salivsekretionen var högre hos snusare (p=0.001) än hos icke tobaksbrukare (2.5 vs 2.2 ml/min) men det var inga statistiska skillnader i buffertkapacitet mellan grupperna. Det förelåg heller inga statistiska skillnader med avseende på plackindex, primär och sekundär emalj- och dentinkaries, DFS eller antal mutansstreptokocker och laktobaciller i saliv. pH-fallet var något mer uttalat bland icke tobaksbrukare än hos snusare (NS). Gruppen med snusare hade ett lägre intag av snacks mellan måltider och ett mindre frekvent intag av kakor (p=0.000). Vad avser gingivalindex hade snusare ett medelvärde ± SD för hela bettet på 20.4 ± 18.2 och icke brukarna 14.4 ± 13.9 (p=0.009); motsvarande värden för regio 13-23 var 14.9 ± 20.6 respektive 7.7 ± 11.9 (p=0.003). Sammanfattningsvis visade studien inga statistiska skillnader i kariesförekomst mellan snusarna och icke tobaksbrukare och endast mindre skillnader avseende övriga studerade kariesrelaterade faktorer.

Introduction

In an epidemiological study, the use of tobacco in relation to socioeconomic factors and dental care habits has been studied in a Swedish population [6]. The results showed that smoking and the use of snuff have changed in Sweden over the past 20 years. The study also revealed that tobacco users differed from non-users in terms of frequency of dental appointments and oral hygiene habits. In another epidemiological study covering the same population, dental caries in relation to smoking and the use of Swedish snus was studied [9]. To summarise, the results indicated that daily smoking and snus use did not coincide with dental caries.

Dental caries is a multifactorial disease associated with several factors, such as the use of fluoride, dietary habits, salivary secretion rate and buffer capacity, plaque amounts and cariogenic micro-organisms [1, 4, 9, 11, 13, 15]. The daily use of fluoridated toothpaste has had a dramatic effect on the decline in caries over the last 40-50 years [2, 21, 25].

The association between dental caries and the use of smokeless tobacco, including snuff and snus, has been presented in reviews and in an epidemiological study with contradictory results [8, 26, 27]. One possible explanation is that the ingredients in smokeless tobacco vary between countries [10, 24]. In a literature review, Lee [14] reported that Swedish snuff products – known as "snus" [7] – differ significantly from other types of smokeless tobacco. For example, they contain lower levels of tobacco-specific nitrosamines (TSNA), only minor amounts of fermentable carbohydrates and have higher pH values than snuff from other countries.

Snus is usually placed under the upper lip in close contact with the tooth surfaces and is kept in place for a long period of time. One Swedish study reported that almost 50% of regular snus users stated that they used snus for 10-18 hours a day [22]. This makes this product interesting from a cariological point of view. The aim of the present study was to investigate some caries-related factors and their effects on dental caries in a group of adult habitual snus users and to measure the pH fall in dental plaque *in situ* in a subgroup after a sucrose rinse.

Material and methods

Study population

The present study was carried out between 2009 and 2011. The ethical regulations for research, specified in the Declaration of Helsinki (5), were followed. The Ethics Committee at Karlstad University approved the study (dnr. 2008:29). Informed consent was given by the participants before the study began. They were given oral and written information about the purpose and design of the study.

The subjects comprised males and females between 26 and 62 years of age (n=102), all living in the city of Karlstad or its vicinity, recruited among individuals with different jobs and different educational levels. The main inclusion criteria were that they were healthy, non-smokers and daily snus users for ≥ 10 years. Most of the snus users (n=66) consumed less than one tin/day, 26 used one tin/day and eight snus users more than one tin/day (1 tin corresponds to around 24 g snus). Sixty individuals had been snus users for more than 20 years and 37 of these for more than 25 years.

Control group

The inclusion criteria for the control group were that they were healthy, with no tobacco use for ≥ 10 last years. They were selected to be similar to the test group in terms of age, gender and educational level. The final control group (n=101) consisted of 29- to- 61-year-old individuals. In order to recruit these 203 individuals, a total of 295 individuals were contacted; there were therefore 92 non-respondents.

Drop-outs

Two subjects among the snus users were excluded from the study because they were too old to participate or developed illness. As a result, 201 (100 snus users and 101 non-users) subjects ultimately participated in the study, 188 (93%) of whom were males. There were two missing samples relating to the measurement of the salivary secretion rate among snus users and two relating to buffer capacity among non-users.

Questionnaire

All the participants were asked to complete a questionnaire in conjunction with the clinical examination. It contained 32 questions about medical health, educational level and the use of tobacco, dietary habits and oral care habits. Educational level was described as low education (9-year compulsory school, practical upper secondary school) or higher education (upper secondary school, college of higher education) [6].

Clinical examination

All the clinical examinations were carried out by one of the authors (LH) at a modern, fully equipped

dental office, using a mirror and probe with optimal light conditions. The clinical and radiographic examination of each individual took approximately 60-90 min.

Clinical caries registration

The number of teeth, as well as the number of decayed and filled tooth surfaces (DFS), was recorded; the third molars were excluded. All the tooth surfaces available for clinical evaluation were examined for caries, according to the criteria described by Koch (12). *Initial caries* = loss of mineral in the enamel, giving a chalky appearance but without any clinical cavitations; *manifest caries* = new carious lesions on surfaces not previously restored and of such an extent that they could be verified as cavities by probing and, when probing fissures using light pressure, the probe became stuck; *secondary caries* = lesions according to the criteria for manifest caries but on restored tooth surfaces.

Radiographic caries registration

Four bitewing radiographs, two on each side, were taken on all participants. They were mounted in frames and subsequently examined using observation binoculars according to Mattsson (18). All the tooth surfaces that could not be evaluated clinically were evaluated from the radiographs. Dental caries on the radiographs was recorded as follows: *initial caries* = the lesion involved enamel but did not involve the dentine; *manifest caries* = lesions extending into the dentine (12).

Salivary and microbiological analyses

The participants were instructed not to eat, drink or use snus for one hour before sampling. Paraffin-stimulated whole saliva was collected in a graded cylinder and expressed as ml/min. Buffer capacity was estimated by means of a chairside test (CRT Buffer; Vivacare, Schaan, Liechtenstein). One millilitre of the saliva was transferred into a vial with 5.7 ml of transport medium and sent to the Department of Cariology at the University of Gothenburg for an estimation of the numbers of mutans streptococci (MS) and lactobacilli (LB) on selective agar media (1).

Registration of plaque and gingival status

The presence of visible plaque was recorded on six surfaces on each tooth after drying with compressed air, according to the criteria for the plaque index (PLI) grades 2 and 3 (23). The presence of gingival inflammation (GI) grades 2 and 3 was assessed at six sites around each tooth. Gingival inflammation was registered if there was bleeding on gentle probing (17).

Cariogram

A caries risk assessment was made using the Cariogram computer program (3). Information about nine caries-related variables (caries experience, related diseases, dietary content, intake frequency, plaque index, number of mutans streptococci, fluoride programme, salivary secretion rate and buffer capacity) was entered into the program, after which the "chance of avoiding caries" was calculated as a percentage (%).

Measurement of plaque pH

Twenty male subjects, 10 snus users and 10 non-users, were randomly selected from the final 201 subjects. They came to the laboratory for a one-hour appointment (non-users) or for a two-hour appointment (snus users). They were asked not to brush their teeth for three days prior to the test and not to eat, drink or use snuff for two hours prior to the test. Plaque pH was measured *in situ* with a microtouch electrode (Beetrode; NMPH-1, W.P. Instruments Inc., Sarasota, FL, USA) (7, 16), connected to a pH/ISE meter (Orion SA 290A/720, Orion Research, Boston, MA, USA). The electrode was calibrated against standard buffers at pH 7.00 and 4.00 for verification of the electrode slope and function, before and during each test session. Measurements were made at three approximal sites in the upper jaw; one in the premolar region and two in the front region (mesial 15, distal and mesial 13). A salt bridge was created by the subject dipping one of his/her fingers into a beaker with a 3 M KCl solution into which a reference electrode (MERE 1; W.P. Instruments Inc.) was also placed. Plaque pH was measured before and on nine occasions up to 45 min after a one-minute mouth rinse with 10 ml of a 10% sucrose solution. It was carried out twice in the 10 snus users. Directly after the first test, one portion of snus was placed under the upper lip in regio 13. The subjects then rinsed again with sucrose, after which the pH was followed at the three sites for another 45 min.

Statistical analysis

The data were analysed using the Statistical Package of Social Sciences (SPSS). The mean values were estimated and calculations of statistical significance

between variables and groups were made using chi-square analyses. The numbers of MS and LB were transformed to logarithms before the statistical analysis. The differences between snus users and non-users were calculated using Student's *t*-test. Statistical significance was defined as $p < 0.05$. Twenty randomly selected bitewing radiographs were checked twice for approximal caries, using Cohen's kappa. The intra-examiner agreement was 0.81.

Results

The age of the snus users was 42.3 ± 8.7 (mean±SD), while that of the non-users was 42.3 ± 8.3 years. They had visited a dentist for an oral examination 1.9 ± 2.4 and 1.5 ± 2.2 years ago respectively. All the participants but one stated that they used fluoride toothpaste daily or twice daily. Among the snus users, 78 individuals had "high" and 22 "low" educational levels. The corresponding figures among non-users were 83 and 18 respectively. Table 1 describes the number of participants, PLI, GI, DFS, initial and manifest caries, as well as the numbers of MS and LB in saliva. The numerical mean difference for teeth was small between snus users and non-users (27.1 vs 27.5). For DFS, there was no statistically significant difference between the groups, either for the whole dentition or for the upper front area (teeth 13-23). There were no statistically significant differences between the groups regarding initial and manifest caries. The numbers of tooth surfaces with secondary caries were 1.0% of restored tooth surfaces for snus users and 0.9% for non-users.

In terms of GI, snus users had significantly higher values for the whole dentition (p=0.009), as well as for the upper front area (teeth 13-23; p=0.003). For PLI, there was no statistically significant difference between the two groups.

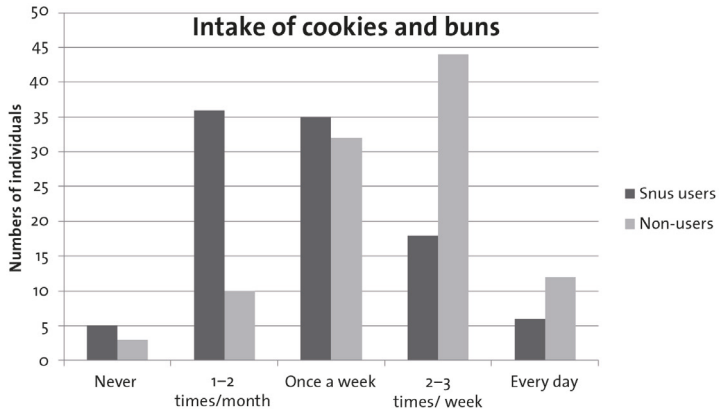
The mean salivary secretion rate for snus users (n=98) was 2.5 ml/min and for non-users 2.2 (n=101) ml/min, which was statistically significant (p=0.005).

As regards buffer capacity, 84 snus users had a high capacity and 16 a low capacity, while the corresponding values for non-users (n=99) were 86 individuals with a high capacity and 13 with a low capacity respectively (p=0.566). For the numbers of MS and LB in saliva, there were no statistical differences between snus users and non-users.

There was no statistically significant difference between snus users and non-users regarding tooth-brushing habits and approximal cleaning with toothpicks and an interdental brush (data

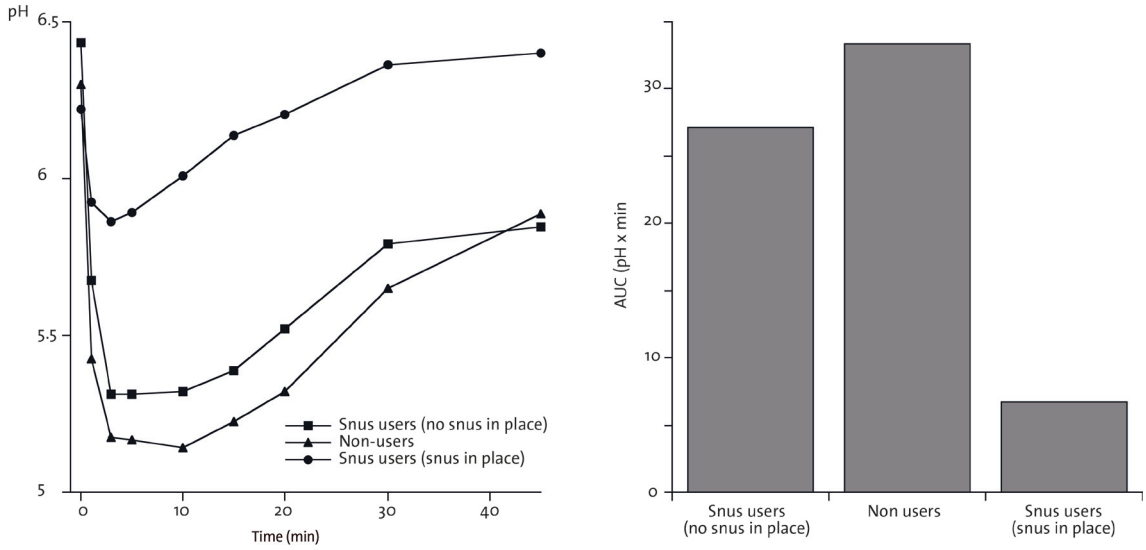
Table 1. Mean and ±SD of plaque index (PLI), gingival index (GI), enamel (initial) caries and dentine (manifest) caries, DFS, mutans streptococci (MS), lactobacilli (LB). Number of snus users = 100 and number of non-users = 101. The p-values indicate comparisons between the snus users and non-users.

	Mean (SD)	p-value		Mean (SD)	p-value
PLI (%)			**PLI upper front (%)**		
Snus users	24.9 ± 18.5	0.084		12.6 ± 17.7	0.070
Non-users	20.7 ± 16.0			8.4 ± 15.0	
GI (%)			**GI upper front (%)**		
Snus users	20.4 ± 18.2	0.009		14.9 ± 20.6	0.003
Non-users	14.4 ± 13.9			7.7 ± 11.9	
Initial caries			**Manifest caries**		
Snus users	3.50 ± 3.83	0.264		0.24 ± 0.70	0.406
Non-users	3.00 ± 3.94			0.33 ± 0.78	
DFS			**DFS upper front**		
Snus users	21.9 ± 16.4	0.648		1.5 ± 2.9	0.762
Non-users	22.9 ± 14.6			1.4 ± 2.8	
MS			**LB**		
Snus users	4.7 ± 1.1	0.436		3.4 ± 1.2	0.054
Non-users	4.6 ± 1.2			3.7 ± 1.2	



◉ **Figure 1.** Intake of cookies and buns in snus users and non-users.

◉ **Figure 2.** Mean plaque pH and area under the curve (AUC) after rinsing with 10% sucrose in snus users and non-users (10 subjects/group). The snus users repeated the sucrose rinse with a portion of snus present in the mouth.



not shown). In contrast, the use of dental floss was more frequent among non-users (p=0.001). A larger number of snus users (n=23) than non-users (n=10; p=0.049) did not eat snacks between meals. Fewer snus users (n=18) stated that they consumed cookies and buns two to three times a week as compared with the non-users (n=44) (Fig. 1) and they also had a lower daily intake between main meals (n=6 vs 12; p=0.001). There was no significant difference in the intake of candy, sweets and soft drinks between the two groups (data not shown).

The mean Cariogram value, expressed as the "chance of avoiding new lesions in the near future" was 65.4 ± 16.2% for snus users and 64.5 ± 16.4% for non-users (NS). The measurement of plaque pH showed that non-users had a somewhat more pronounced pH drop than snus users (Fig. 2), but this difference (based on AUC; area under the curve) was not statistically significant. When the users placed a portion of snus under the lip and then rinsed with sucrose, the pH fall was smaller than when no snus was present in the mouth. This holds both for users and non-users (p=0.001, Fig. 2.)

Discussion

It appears to be generally believed among the public and among dental personnel in Sweden that snus may protect the user from caries, but, to our

knowledge, no scientific support for this opinion has as yet been presented. More research is therefore needed and the aim of the present investigation was to examine caries and some caries-related factors in a group of middle-aged habitual snus users and to measure the pH fall in dental plaque *in situ* in a subgroup after a sucrose rinse. This is one part of a series of studies of oral health and the use of tobacco, focusing on Swedish snus users and on various snus products, which is being carried out by our research group (6, 7, 9).

The main findings in the present cariological study are that there appear to be only minor differences between snus users and non-users in the studied caries-related factors. There may be several explanations for this. One is that almost all the participants used fluoride toothpaste every day, which may mask a caries effect of snus. Another reason could be that the studied population had a relatively low caries risk. Moreover, studies (19, 20) have shown that sociodemographic factors, such as "high" educational levels, as in this survey, may mask the effect of the use of snus. Most participants had a large number of remaining teeth, which indicates that they are attentive to their oral health. Our previous study (6) showed that tobacco users did not brush their teeth as frequently as non-tobacco users. In the present study, however, there was no statistically significant difference between the groups in terms of tooth-brushing habits, although non-users flossed more frequently than snus users. It has also been found that tobacco users do not visit a dental clinic as often as non-users (6), which is in agreement with the present findings.

Measurements of the number of cariogenic micro-organisms were included in the present study as indicators of caries risk. There was no statistically significant difference between snus users and non-users in relation to either MS or LB, which is in agreement with our previous findings (9). That study also showed that the salivary secretion rate did not differ between snus users and non-users. This is in contrast to the present study, which showed that snus users had a higher secretion rate than non-users. However, the numerical difference between the groups was relatively small (2.5 vs 2.2 ml/min) and both secretion rates are regarded as high.

The results of the plaque-pH measurements after a sucrose rinse showed a tendency towards a somewhat more pronounced pH drop among non-users than snus users, although the difference for the AUC was not significant. One interesting observation in this respect for the snus users was that the presence of snus in the mouth during rinsing with sucrose inhibited the pH fall. This goes hand in hand with our recent findings showing that Swedish nicotine-containing snus products increase the plaque pH (7).

The results of the Cariogram data, a multifactorial risk assessment model (3), showed no difference in the "chance of avoiding caries in the future" between snus users and non-users. This supports the clinical data indicating that the daily use of snus neither increases nor decreases the risk of caries in the studied population. However, more studies are needed in other age groups, which may run a high risk of developing dental caries.

To conclude, this clinical study revealed no statistically significant difference in caries prevalence between the group's snus users and non-users and there were only minor differences regarding caries-associated factors.

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