ISSN 2219-6749

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The Journal of Forensic Odonto-Stomatology

Volume 21, n. 2 - Dec 2003

OFFICIAL PUBLICATION OF THE
INTERNATIONAL ORGANIZATION FOR
FORENSIC ODONTO-STOMATOLOGY

CONTENTS

ISOLATION AND GENOTYPIC COMPARISON OF ORAL STREPTOCOCCI FROM EXPERIMENTAL BITEMARKS

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ABSTRACT

The feasibility of recovering and genotypically comparing oral bacteria from bitemarks for forensic purposes was assessed experimentally. Volunteers firmly bit their own upper arms and bitemarks were sampled at intervals to recover viable *Streptococcus* isolates. The recoverability of bacteria decreased over time but an average of more than one thousand viable organisms was recovered 24 hrs after biting, provided the site remained relatively undisturbed. Physical exertion, manual rubbing and application of moisturizing lotion all decreased bacterial recoverability compared to controls. Streptococci could also be recovered from bites inflicted on various fabrics. Genomic profiles (DNA "fingerprints") of bacteria recovered from bitemarks could be identified exclusively with those from the teeth of the individual responsible. These findings suggest that a bacterial genotyping approach to bitemark analysis could have forensic application in situations where the perpetrator's DNA cannot be recovered from an oral contact site. **(J Forensic Odontostomatol 2003;21:23-30)**

Key words: DNA typing, bitemark, oral bacteria, genotype, *Streptococcus*.

INTRODUCTION

Sexually abusive crimes against women and children have been reported with increasing frequency over the last two decades[1] and estimates indicate that between 9% and 24% of women will be assaulted at least once in their lifetime.[2] Bitemarks are associated with both attempted rape and child abuse, and forensic examination of human bitemarks is often a central issue in the identification of the perpetrators of such crimes. Bitemarks, however, are complex injuries involving a number of factors and can be very difficult to analyze,[3] interpretation frequently requiring a degree of subjective judgment which is often challenged.[4] Nevertheless, as biting may be the principal forensic evidence of such attacks, all opportunities to acquire information from such lacerations should be explored. Advances in molecular biological techniques now offer a further dimension to forensic analyses but because human saliva and skin secretions contain nucleases, recovery of human DNA fragments from recent bitemarks may not always be successful.[5]

The human oral cavity maintains a large and varied community of bacteria, many of which are unique to this habitat.[6] The predominant genus is *Streptococcus* which includes several benign species[7] universally present in the human oral cavity and which express adhesins mediating attachment to the salivary macromolecules selectively adsorbed onto the tooth surface.[6] These adhesins facilitate re-colonization of the tooth surfaces within minutes of professional cleaning.[8] Because these bacteria are found on all tooth surfaces, even on those not prone to plaque accumulation such as the incisors,[6] streptococci are likely to be initially present in essentially all bitewounds inflicted by humans. The oral streptococci are genotypically extremely diverse which has limited attempts to speciate and classify this group of bacteria, even by modern molecular

methods. For example, Alam *et al.*,[9] compared 72 isolates by randomly-primed polymerase chain reaction (PCR) and found no two strains with more than 90% similarity. Other PCR-based studies have further emphasized the genotypic variety of the oral streptococci.[10,11] Whereas this diversity has hampered identification and taxonomy of these bacteria it may prove advantageous for forensic purposes.

The aims of this study were: (i) to assess the practicability of recovering oral streptococci from bitemarks inflicted on human skin and clothing; and (ii) to evaluate the feasibility of matching *Streptococcus* isolates recovered from bitemarks with those recovered from the incisor teeth of the perpetrators, using a genomic comparison (DNA "fingerprinting") approach.

MATERIALS AND METHODS

Healthy volunteers bit themselves in the biceps region of the upper arm, maintaining as much pressure as they could tolerate for 10 seconds. This resulted in an imprint lasting for at least ten minutes and often produced mild bruising. Each bitemark was sampled only once and, if not sampled immediately, was covered by the volunteers' clothing until the appropriate time. No sites were bitten twice in the same day and sampling times were arranged such that at least one hour elapsed between each bite inflicted by the same individual. This was to ensure an adequate bacterial load for each bite. Participants were directed not to undertake any strenuous activity nor to interfere with the bitemark (unless specifically directed) between the time of biting and the time of sampling. Bacteria were recovered from bitemarks by swabbing the area with a sterile cotton-tipped applicator moistened in sterile tryptic soy broth (TSB).* The swab was placed in 5 mL of sterile TSB and vortexed vigorously for 1 minute to dislodge bacteria. The suspension was serially diluted in TSB and 100 µL volumes plated onto Mitis-Salivarius (MS) agar* plates which were then incubated at 37°C under anaerobic conditions (10% [v/v] hydrogen, 5% [v/v] carbon dioxide in nitrogen) (MS agar is selective for streptococci[12]). Four days later, the agar plates were removed from the anaerobic atmosphere, the bacterial colonies enumerated and the number of colony-forming units recovered by the swab, calculated.

The initial trial determined the length of time following biting that viable bacteria could be recovered from bitemarks. Subsequently, various treatments of the bitemark area, which we surmised might adversely affect bacterial recoverability, were assessed by variations of the above method. To determine the influence of preservative-containing moisturizing lotion, volunteers applied Nivea® Body cream† to the upper arm one hour prior to biting; the bitemark was sampled for bacteria three hours following biting. As a natural reaction to biting, fresh bitemarks were briefly rubbed manually immediately following biting, to the extent that the participant gained some degree of perceived relief from the infliction, and the bitemarks sampled three hours later. The effect of brief physical exertion was assessed by requiring the participants to run on a treadmill for ten minutes at 75% of their age-predicted maximal heart rate[13] immediately following biting; bitemarks were sampled three hours later. To assess bacterial recoverability from various fabrics, a freshly laundered square (approximately 15 cm^2) of fabric was pinned to the volunteer's shirt-sleeve and the bite inflicted through the fabric square. Five fabric types were tested for each of six participants. The volunteers wore the squares until the appropriate sampling time when the squares were removed, immersed in 100 mL of 0.3% TSB and agitated (230 rpm) for five minutes on an orbital shaker.‡ Dislodged bacteria were collected by passing the TSB through a 0.45 µm cellulose nitrate filter§ and the filter then vortexed in 3 mL of TSB. A sample of the TSB was subsequently diluted and plated as above.

Bacterial strains from eight volunteers were compared by whole genomic "fingerprinting" according to the following method. Bacterial cultures from bitemarks (six hours old) and from the lower incisors were examined under a dissecting microscope.¶ At least ten colonies (all less than 2 mm diameter) were isolated from the two sites from

* *Difco, Becton Dickinson Co., Sparks, MD.*
† *BDF Beiersdorf, AG Hamburg, Germany.*
‡ *Queue Systems, Parkersburg, WV.*
§ *Sartorius, Goettingen, Germany.*
¶ *SZ-CT, Olympus, Japan*

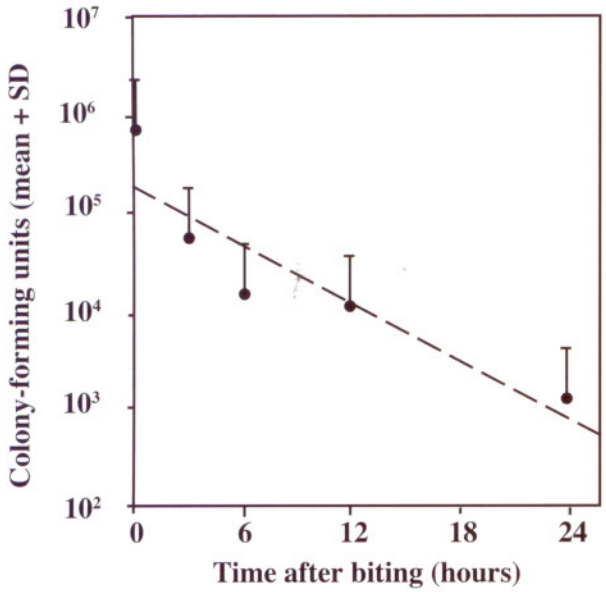

Fig.1: *Recovery of viable oral streptococci from bitemarks inflicted on the upper arms of 13 volunteers (mean + standard error). Bacteria were recovered and enumerated at intervals by swabbing and plating onto Mitis-Salivarius agar. The exponential line (dashed) was fitted by CA-Cricket Graph III (Computer Associates, Vancouver, Canada).*

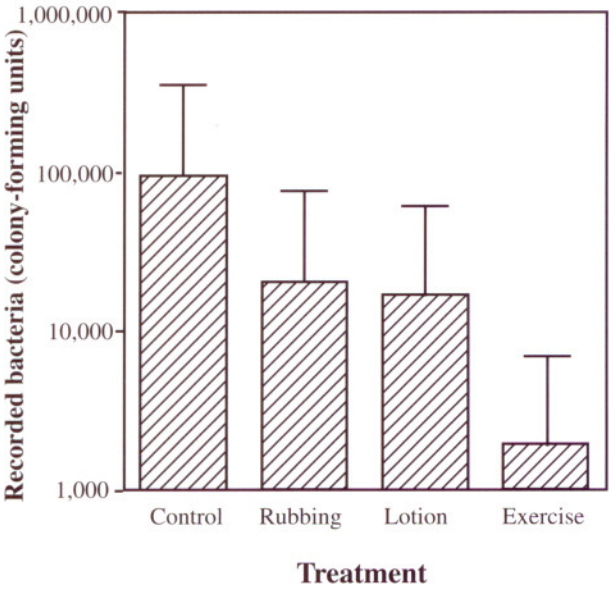

Fig.2. The effects of (1) brief manual rubbing, (2) commercially available moisturizing lotion, and (3) physical exercise, on recovery of oral streptococci from self-inflicted bitemarks inflicted on the upper arms of ten volunteers. The control bites were inflicted on untreated sites and remained undisturbed until sampling. Bacteria were recovered and enumerated three hours after biting by swabbing and plating onto Mitis-Salivarius agar.

each participant by re-streaking onto MS agar. Overnight cultures of purified bacterial strains, grown in brain-heart infusion (Difco) supplemented with 0.5% yeast extract (BHI-YE), were used to inoculate pre-warmed BHI-YE (8 mL) which was incubated at 37°C until reaching an absorption (A540) ≈ 0.4. Solid glycine (0.5 g) was added and incubation continued for a further 45 minutes. Cultures were cooled in iced water and the bacterial cells washed and lysed by the method of Macrina *et al.* [14] The released DNA was then purified and concentrated according to the procedure of Marmur.[15]

Several restriction endonucleases (*Eco* R1, *Hind* III, *Hae* III, *Sac* II, *Not* I, *Eag* I, *Nde* I, *Sal* I, *Pvu* II) were considered for DNA digestion but, using a test strain of *Streptococcus gordonii*, *Pvu* II* was chosen as it produced a greater number of clearly resolved larger sized fragments. Purified chromosomal DNA (≈ 500 μg/mL) was digested with *Pvu* II (200 unit/mL) in NE2 buffer* by incubating at 37°C for 2 hours. The DNA fragments were separated by electrophoresis at 40V through an agarose gel (0.5%) for approximately 5 hrs using 40 mM Tris acetate buffer (pH 8.0) containing 2 mM Na_2EDTA.[16] Gels were calibrated with a *Hind* III digest of lambda phage DNA.† DNA digests of strains isolated from the teeth and bitemarks of the same individual were always compared on the same gel.

Gels were stained for 10 minutes with aqueous ethidium bromide (5 μg/mL) and destained in distilled water for 15 minutes before photography with ultraviolet trans-illumination. The DNA fragment patterns of bacterial isolates were compared visually. Participants in this study were not undergoing antibiotic therapy and were not using antiseptic mouth rinses. The involvement of human participants was approved by the Medical College of Georgia Human Assurance Committee and by the University of Otago Ethics Committee.

* *New England Biolabs, Inc. Beverley, MA.*
† *Life Technologies, Grand Island, NY.*

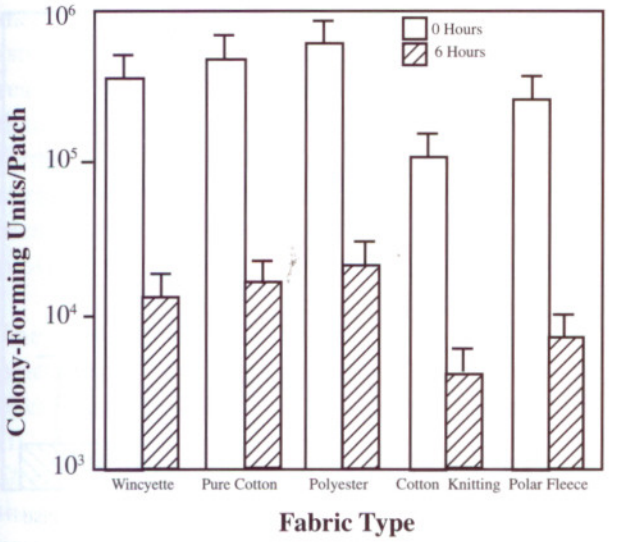

Fig.3: Recovery of oral streptococci from various types of fabric following biting. Bacteria were recovered by agitating the fabric in TSB, concentrating by filtration and plating onto Mitis-Salivarius agar.

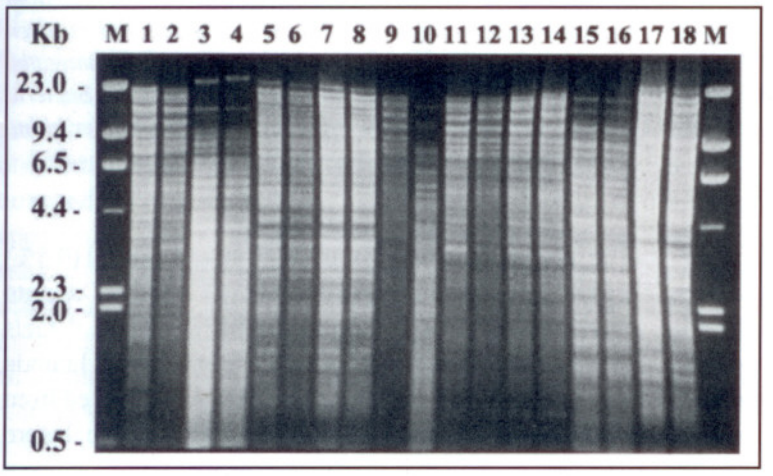

Fig.4: DNA profiles of bacteria isolated from the incisors and bitemark from one subject. The first and last lanes (designated M) contain the Lambda phage DNA calibration markers. Odd numbered lanes contain digested DNA from incisor isolates and even numbered lanes digested DNA from bitemark isolates.

RESULTS

Oral streptococci recoverable from the skin following biting decreased exponentially over time (Fig 1). The average number of colonies recoverable after 24 hrs was slightly more than one thousand, provided that the skin remained relatively undisturbed (Fig. 1). The decrease over the first three hours was 30.8%/hr but this was reduced to 5-6%/hr between hrs 6 and 24. Samples taken from control (unbitten) sites of each of thirteen participants failed to produce more than two colonies/site cultivable on MS agar and ten of the thirteen control samples produced no colonies, indicating negligible background contamination.

Manual rubbing of the freshly bitten sites resulted in a decrease of approximately 80% in recoverable bacteria (Fig. 2). Similarly, the pre-application of moisturizing lotion containing various solvents, oils and preservatives, caused a decrease in recoverable streptococci of just over 80% (Fig. 2). Moderate physical exertion for ten minutes was even more detrimental, resulting in a decrease in recoverable bacteria of more than 95% (Fig. 2). However, despite large proportional decreases in bacterial numbers following exercise, greater than a thousand colonies could still be recovered from most subjects (Fig. 2) which would be more than required for genotypic analysis. All tested fabric types retained viable oral streptococci over the six-hour period examined though the recoverable number of organisms decreased by more than 90% when compared to the initial sample (Fig. 3). Streptococci were not recovered from control fabric squares that had not been bitten.

From eight volunteers, the DNA "fingerprints" (genotypes) of 58 streptococcal isolates from the teeth and 54 from the bitemarks were compared. A total of 60 distinct patterns were identified. Between 2 and 8 genotypically distinguishable strains were isolated from each dental source and also from each bitemark (Table 1). A minimum of 1/6 and a maximum of 4/4 bitemark genotypes could be matched with a genotype from the corresponding incisors (Table 1). No bacterial genotypes could be matched between individuals (Table 1). An example of a genomic DNA gel comparing bacterial isolates from the bitemark and incisors is shown in Fig. 4.

A frequent problem encountered in the development of this procedure was the difficulty of successful subculturing from the primary isolation plate. Although we aimed to recover at least ten strains from

Table 1: *Genotypic comparison of oral streptococci recovered from bitemarks and from the teeth responsible for inflicting the bite.*

	Participant															
	1		2		3		4		5		6		7		8	
	Number and source of isolates[1]															
	B	T	B	T	B	T	B	T	B	T	B	T	B	T	B	T
genotype a	2	2	2	2	3	3	3	3	1	2	4	1	1	2	2	3
genotype b	1	1	2	2	1	1	2	3	1	0	1	2	1	0	1	1
genotype c	1	1	1	1			1	1	1	0	1	0	1	0	1	1
genotype d	1	1	1	1			1	0	1	0	1	0	1	0	1	1
genotype e	1	1	1	1			1	0	1	0	1	0	0	1	1	0
genotype f	1	0	1	1			0	1	1	0	0	2	0	1	0	2
genotype g	1	0	1	0					0	1	0	1	0	1	0	1
genotype h	1	0	0	1					0	1	0	1				
genotype i	0	1							0	1	0	1				
genotype j	0	1							0	1						
genotype k	0	1														
Number of genotypes from bitemarks	8		7		2		5		6		5		4		5	
Number of genotypes from incisors	8		7		2		4		5		6		4		6	
Matching bitemark genotypes/total genotypes[2]	5/11		6/8		2/2		3/6		1/10		2/9		1/7		4/7	
Matching bitemark isolates/examined bitemark isolates[3]	6/9		8/9		4/4		6/8		1/6		5/8		1/4		5/6	

(1) For each of eight study participants, the number of isolates of each genotype (designated a, b, c, etc.) recovered from the bitemark (B) and from the teeth (T) is shown. Note that genotypes designated a, b, c, etc. from one subject were distinct from those with the same letter designation isolated from all other subjects.
(2) The number of genotypes recovered from the bitemark that were indistinguishable from a genotype recovered from the teeth/total number of genotypes (from both teeth and bitemarks).
(3) Number of isolates recovered from the bitemark that were genotypically indistinguishable from a tooth isolate/number of examined isolates from the bitemark.

both the bitemark and the tooth primary isolation plates, failure either to subculture several strains or to recover sufficient DNA for endonuclease digestion resulted in fewer DNA profile comparisons (Table 1).

DISCUSSION

The various species of non-pathogenic streptococci inevitably comprising the human oral microbiota are rarely found in other environments such as the skin.[6] Recovery of oral streptococci from the skin (or other surfaces) would therefore seem to imply contact with either oral surfaces or saliva (which may in itself provide compelling evidence of oral involvement in a nondescript laceration). As these organisms are not adapted to live on the skin, their survival in this environment is limited. Nevertheless, under favourable conditions, streptococci were recovered from bitemarks in large numbers for up to 24 hrs after biting. On average, women over the age of 20 yrs seek medical assistance seven hours after a sexual assault and younger women eleven hours after assault[17] and it should therefore be feasible to recover oral bacteria originating from the perpetrators of most crimes involving biting. Oral bacteria were also recovered from various fabrics from which it may

be difficult to obtain other forensic information. Again the recoverability of bacteria decreased fairly quickly but the nature of the fabric seemed to have little effect on survival and large numbers of streptococci could be recovered from all fabric types.

Conditions associated with the commitment of violent crimes are unlikely to be as conducive to bacterial survival as those of a laboratory investigation. Whereas it is impossible realistically to simulate the stress experienced by the victims of violent crime, a degree of physical activity seems almost inevitable and, as this study demonstrated, brief exertion markedly diminished the survival of oral bacteria in bitemarks. Furthermore, application of moisturizing lotions and the natural response of simply rubbing the afflicted site also reduced bacterial recoverability. The loss of viable bacteria during exercise is probably due to the anti-microbial effects of sebaceous secretions. Other factors that induce sweating, such as a warm humid climate, will almost certainly diminish recovery of oral bacteria from bitemarks also. Nevertheless, even after the most detrimental treatments such as physical exertion, bacteria were recoverable in large numbers.

The current investigation attempted to replicate natural conditions of bacterial deposition as closely as possible by actual biting. Although the number of organisms in the bitemarks could not be standardized, we felt that the fact that bacteria had been deposited authentically was very important. The arms are the second most frequently bitten site after the breasts in assaults,[18] but obvious ethical and anatomical considerations restricted the experimental bite sites. All bitemarks were covered by loose clothing until sampling and this may have enhanced bacterial survival by reducing desiccation. Brown *et al.*[19] performed similar experiments by placing measured volumes of saliva on the chest of a volunteer and were able to recover streptococci for at least six hours with a similar exponential decrease over time.

Elliot *et al.*[20] demonstrated that strains of *Streptococcus salivarius*, the most prominent species in saliva, could be distinguished by pyrolysis mass spectroscopy and advocated recovery of these bacteria from bitemarks for comparison with oral isolates for forensic purposes. However, the technique involves specialized equipment and the interpretation of results is complex,[20] possibly resulting in the method not being widely adopted. The genotypic approach described in the current study involves relatively straightforward and frequently used molecular biological techniques requiring inexpensive apparatus.

Our findings indicate that, under controlled laboratory conditions, it is feasible to match one or more bacterial strains recovered from a bitemark with those from the teeth of the perpetrator. We had anticipated that sampled tooth surfaces would be dominated by two or three strains of streptococci and therefore selected the relatively labour-intensive whole genomic method in preference to a PCR-based approach. PCR may have facilitated analysis of greater numbers of bacteria but at the possible expense of resolution. In fact, there were sometimes as many as eight abundant bacterial colony types in a sample and the dominant genotypes from the bitemark were generally not the dominant strains on the corresponding tooth site. These findings, together with the survival data shown in Fig. 1, suggest that the skin imposes significant challenges to these organisms. Furthermore, microscopically indistinguishable colonies from the two sources from the same individual were often genotypically distinct. Thus strains dominating the tooth surface will not necessarily be prominent in the bitemark and a more extensive comparison of strains will be necessary to evaluate the probability of recovering identical strains from two individuals. Nevertheless our results indicate that development of a microbial genome-based approach has the potential to provide strongly supportive forensic evidence. As streptococci survive for several hours on inanimate surfaces,[21] a genomic identification method could also be applied to support analysis of bitemarks imprinted on materials other than skin.

The application of bacterial genotypic analysis for forensic purposes cannot indisputably link a suspect to a crime because the genetic material is not the suspect's. The likelihood of recovering the perpetrator's own genetic material from a bitemark is diminished by the presence of nucleases in saliva which rapidly degrade naked DNA.[22] Sweet *et al.*[23] have attempted to overcome this problem by recovering intact epithelial cells deposited in the bitemark (by the aggressor) and amplifying the DNA

protected within the cell. Identification using the perpetrator's own DNA is, potentially, an almost infallible forensic aid but even under controlled experimental conditions, more than 20% of attempts to amplify salivary DNA deposited on the skin of cadavers (presumably maintained at 4°C) were unsuccessful.[23] Under field conditions the success rate is likely to be less. Therefore the bacterial approach described has the potential to provide supportive evidence and a valuable back-up measure.

To be of value in a courtroom, it will be necessary to determine the frequency with which bacterial strains of indistinguishable genotype occur among the human population (which we cannot do from the data currently available). This may be significant among siblings[24] and perhaps close relatives but among unrelated individuals there could be a statistically sound opportunity to obtain supportive evidence from bacterial genotypes. Furthermore, the occurrence of multiple matches among strains from bitemark and tooth site from the same individual (which occurred in six of the eight participants) would further increase the statistical resolution.

In conclusion, this study demonstrates that bacteria, unambiguously derived from the oral cavity, can be recovered from bitemarks impressed on human skin for up to 24 hrs. Because of the extreme genotypic diversity of the oral streptococci, bacteria recovered from bitemarks could be matched exclusively to the teeth of the "perpetrator" in each of eight samples, indicating that a bacterial genotyping approach has the potential to support the identification of the perpetrators of crimes involving biting. We are currently assessing approaches to optimize and expedite molecular identification of streptococcal isolates from bitemarks and teeth as well as examining the long-term genotypic stability of naturally occurring oral streptococcal populations.

ACKNOWLEDGEMENTS

This study was supported by the New Zealand Dental Research Foundation Board, the New Zealand Lottery Grants Board and the Department of Oral Rehabilitation, Medical College of Georgia, (U.S.A.)

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CHRONOLOGICAL AGE DETERMINATION BASED ON THE ROOT DEVELOPMENT OF A SINGLE THIRD MOLAR: A RETROSPECTIVE STUDY BASED ON 2513 OPGs

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ABSTRACT

The aim of this survey was to correlate chronological age with the root development of only one third molar using a sample of 2513 subjects of Belgian Caucasian origin within the age range of late 15 to 22 years. Observations were performed by two observers, who were calibrated for intra- and inter-rater reliability by means of Kappa statistics and each third molar present was scored according to the modified method of Gleiser and Hunt. The method used in this study delivered linear regression formulae based on a sole wisdom tooth, divided according to gender. The results revealed standard deviations similar to those reported in comparable publications and even to those calculated with other skeletal age calculation techniques. **(J Forensic Odontostomatol 2003;21:31-5)**

Keywords: forensic odontology, dental age calculation, third molar.

INTRODUCTION

In forensic sciences age estimation is not only one of the standard requests upon the discovery of a dead body, but is also crucial if identity is in question in living individuals.[1-3]

Age calculation by means of tooth development and particularly the root formation of third molars has already often proved to be effective in determining an individual's chronological age. Other methods for age calculation using skeletal radiology (hand-wrist, sternoclavicular joints, long bones and vertebrae) or secondary sex characteristics, have at least comparable limitations.[4-9]

The highest accuracy in dental age estimation is achieved when an individual's growth is rapid and many developing teeth are present,[9-13] but between the late teens and early twenties only the developing third molars are useful for age calculation. Although some have argued that their variability in morphology, time of formation and time of eruption[3,14] have unfavourable effects on the age calculation,[3,14-19] it is a fact that this view is based on studies of small samples [3,15-16,18] or case reports.[9,20] In a previous retrospective study the correlation of the chronological age of an individual with the dental development of the third molars was investigated and based on a large sample of orthopantomograms (OPG) taken from individuals with a known chronological age.[21] This led to a series of linear regression formulae, based on the number of wisdom teeth present and their exact location in the upper and lower jaws. On the other hand age calculation of individuals presenting only one third molar often meant a problem because of insufficient numbers and therefore the statistical difficulty of obtaining an appropriate regression formula for this category.

In practice third molars are frequently missing[10,22]: the prevalence of agenesis of one wisdom tooth is reported to be 16% [23] and as the dental practitioner is also often confronted with irregular or malformed crowns, impaction or malposition, which makes dental hygiene difficult, irreversible destruction and loss follows.

Table 1: *Absolute and total number of orthopantomograms in each age category for males and females.*

	15y	16y	17y	18y	19y	20y	21y	22y	23y	Total (n)
Males	20	125	103	146	159	174	219	106	3	1055
Females	33	159	162	191	212	250	308	134	9	1458
Total	53	284	265	337	371	424	527	240	12	2513

y = years n = number

Table 2: *Frequency distribution for males and females of the number of third molars (n) present on all 2513 orthopantomograms evaluated.*

	n	Freq	Total
Males	1	34	34
	2	133	266
	3	141	423
	4	747	2988
Females	1	58	58
	2	180	360
	3	181	543
	4	1039	4156
Totals			8828

Table 3: Some examples of the outcome of the Kappa statistics.

	Weighted κ-value	S.E.	95% LL	95% UL
A1UL by **A2**UL	0,79	0,05	0,70	0,89
A1LR by **A2**LR	0,86	0,04	0,79	0,94
B1UR by **B2**UR	0,65	0,09	0,47	0,83
B1LR by **B2**LR	0,82	0,05	0,72	0,91
A1UL by **B1**UL	0,84	0,05	0,74	0,94
A1LR by **B1**LR	0,87	0,03	0,81	0,94

κ-value: Kappa value
S.E.: Standard Error
LL: Lower limit of the 95% confidence interval
UL: Upper limit of the 95% confidence interval
A1: Observer 1, observation 1
A2: Observer 1, observation 2
B1: Observer 2, observation 1
B2: Observer 2, observation 2
UR: upper right
UL: upper left
LR: lower right
LL: lower left

The aim of this study was to correlate the chronological age with the root development of only one third molar.

MATERIALS AND METHODS

In this study the same material as previously used[21] consisted of a large sample of 2513 OPGs from the School of Dentistry, Oral Pathology and Maxillo-Facial Surgery of the Katholieke Universiteit Leuven, Belgium starting from the early 1970s until early 2002.

All screened subjects were from Belgian Caucasian origin within the age range of late 15 to 22 years with no medical history, showing no obvious dental pathology and having at least one third molar.

The frequency distribution of the number of third molars present on each of the 2513 evaluated OPGs (1055 males, 1458 females) (Table 1) emphasizes the scope of the investigated sample, that is the possibility of studying the developmental stages of 8828 wisdom teeth (Table 2). Given an individual with two wisdom teeth, two separate linear regression formulas could be calculated, for each third molar respectively.

Age calculation was performed using the modified method of Gleiser and Hunt[24] which consists of a 10-stage developmental scale, with three stages for crown formation and seven for root development, previously used by Kohler *et al.* (Fig. 1).[22] All of the third molars were given a score according to their development stage, where the least developed root of a wisdom tooth with multiple roots defined the score.

Two observers were calibrated in advance by scoring 50 OPGs twice with an interval of two weeks, followed by inter- and intra-observer reliability tested using Kappa statistics.

Table 4: *Linear regression formulae for males and females based on the presence of only one wisdom tooth*

	3rd M	R^2	Regression formulae	S.D.	σest
Males	UR	0.38	12,2870+0,8169 UR	1,57	1,59
	UL	0,40	12,2353+0,8231 UL	1,53	1,62
	LL	0,40	13,3669+0,7291 LL	1,53	1,53
	LR	0,38	13,6521+0,6998 LR	1,56	1,56
Females	UR	0,35	13,8062+0,6799 UR	1,60	1,60
	UL	0,35	13,8073+0,6759 UL	1,59	1,59
	LL	0,37	14,2041+0,6639 LL	1,55	1,55
	LR	0,39	14,0809+0,6770 LR	1,54	1,54

3rd M: third molar present
R^2: root square **(linear correlation coefficient)**
S.D.: standard deviation
σ_{est}: standard error of the estimate

$$\sqrt{\frac{\sum(X-X')^2}{N-2}}$$

X': predicted score
X: actual score
N: number of observations

UR: upper right
UL: upper left
LR: lower right
LL: lower left

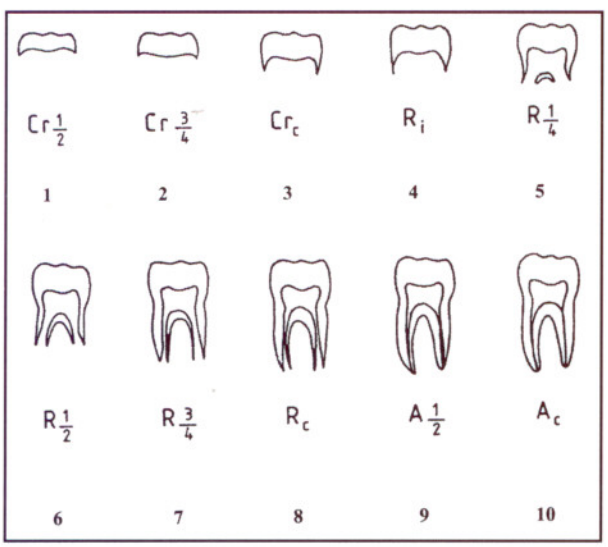

Fig.1: *Development stages according to a modification of the technique by Gleiser and Hunt*[22]

Linear regression analysis was applied in order to obtain linear regression formulae for dental age estimation, with the chronological age as the independent variable and the development stage of only one third molar as the dependent variable, without taking into account the total number of wisdom teeth. This gave four linear regression formulae, one for each of the four third molars.

RESULTS

The Kappa statistics revealed no significant intra- or inter-observer effects (Table 3).

Linear regression analysis led to separate formulae for males and females with the development stage of a sole third molar as the variable (Table 4).

R^2 varied for males from 0,35 to 0,39 and for females from 0,38 to 0,40 and standard deviations for males ranged from 1,53 to 1,57 years, while for females the range was from 1,54 to 1,60 years. The standard error of the estimate ranged for males from 1,53 to1,62 and for females from 1,54 to 1,60 (Table 4).

DISCUSSION

The calculation of these models creates not only the possibility of age estimation of an individual with only one third molar present, but also avoids statistical interference based on multicollinearity between contralateral third molars or antimeres.

The approach of using only one third molar as a variable even though all four can be present has not been encountered in literature. Harris and Nortje[18] had a similar, but not identical method with their measurement of mesial root length of the lower right third molar only and Thorson[3] applied Demirjian's scoring system[11,13] on the lower left third molar, but in cases of agenesis the contralateral was gauged. Other authors,[14,19,22] basing their computations on the presence of four third molars, do not mention the practical consequences when lacking one or more of them, with the exception of Willershausen,[25] whose results are not applicable in the absence of more than one third molar.

Mesotten *et al.*[26] and Gunst *et al.*[21] produced a set of multiple regression formulae from which only the one should be used which applies to the third molar being evaluated. For instance, specially developed multiple regression formulae are reported in cases of the presence of two, three or four third molars. Since high Pearson correlations were found between both upper and both lower third molars, and also, but less so, between antimeres, special measures had to be taken for reasons of multicollinearity.

The weakness of the results was that in cases of the presence of only one third molar , no adequate regression formula could be obtained, because of the relatively small number of individuals in the total sample of the study with only one third molar present. It is now possible to calculate dental age based on a single third molar.

The standard deviations reported in this study have been found to be comparable and approximately equivalent to previous ones[21] and generally in the field of chronological age estimation are very acceptable. The great advantage now is that regression formulae are available for those cases when only one third molar is present, and this is not an infrequent occurrence.

CONCLUSION

The present analysis allowed for the calculation of regression formulae for dental age calculation based on the root development of only one third molar for males and females. Standard errors of the estimate within the range of, respectively, 1,53 to 1,62 and 1,54 to 1,60 were found.

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ORAL HEALTH STATUS OF DRUG ADDICTS IN THE CZECH REPUBLIC

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ABSTRACT

Over the last decade, illicit drug use has become a very pronounced problem in the Czech Republic. Some branches of medicine such as psychiatry, internal medicine and neurology have tried to confront the situation by joint treatment of addicted patients. Dentistry has so far remained outside this multi-disciplinary care despite the fact that the status of dental and other tissues in the oral cavity influences general health and *vice versa*. The aim of the survey on the oral health of drug addicts that was conducted in the Czech Republic from 2000 to 2002 was to collect data to help dental professionals contribute to the complex care of drug addicts. The number of addicted persons examined was 400 and the subgroup of drug addicts consisted of 217 subjects (mean age 23.87 yrs, SD 6.70), all being treated in long term hospitals. Among other oral health features observed, the DMFT and CPITN indices are presented in this paper. The results show severe dental and periodontal tissue destruction in young addicts and two case reports are included. The above survey indicates that dental treatment should become a part of standard care for addicted patients in long-term hospitals. Furthermore, if severe oral tissue impairment in young persons whether in dental practice or during autopsy is encountered a drug addiction should be suspected. If so, the treatment of the person or examination of the dead body should accordingly be adjusted.
J Forensic Odontostomatol 2003;21:36-9)

Keywords: Oral epidemiology, oral health status of drug addicts, DMFT, CPITN, semi-lunar caries

INTRODUCTION

Following the change in the political system in 1989, the Czech Republic has become both a transportation route and a target market for drugs which have become easily accessible and resulted in an increased abuse of drugs.[1] In addition, the misuse of alcohol has increased.

Medical professionals have responded to this situation by promoting complex care for those trying to be rehabilitated and get back to normal life. Several branches such as psychiatry, internal medicine and neurology have joined in this common effort and a methadone program has been implemented to help some addicts in their re-socialization. Dentistry has unfortunately so far been left outside this complex of care, despite the fact that the status of dental and other oral tissues is closely related to the general state of health where dental caries, apical infections, gingivitis and periodontitis can impair dietary intake because of discomfort and pain. As a result a project was initiated in the year 2000 to monitor the oral health of drug and alcohol addicted persons with the aim to collect data on the oral health of drug and alcohol abusers in the Czech Republic and thus establish a database in this field. Moreover, it aimed to promote the inclusion of dental treatment as part of the multi-disciplinary therapy developments and to prevent further destruction of dental and other oral tissues.[2] When the project began, only the most severe oral health problems, such as acute pain or other diseases affecting overall health were treated.

METHODS

In the years 2000-2002, 400 drug and alcohol dependent subjects from long-term hospitals in all regions of the Czech Republic were examined but in this report only data of 217 drug addicts with a mean age of 23.87 (SD 6.70) are presented. The addicted persons were examined by two dentists and the

Table 1: *DMFT values of individual age groups with different duration of addiction.*

Age Groups	DT		MT		FT		DMFT		Duration of addiction	Mean number of erupted teeth per person
	mean value	SD	mean value	SD	mean value	SD	mean value	SD		
15-19	2,77	3,43	0,14	0,41	3,60	3,20	6,51	4,37	4,71	28,29
20-24	3,57	3,87	0,73	1,28	4,63	3,64	8,93	5,45	6,21	30,69
25<	3,67	3,45	2,99	5,26	6,13	4,57	12,79	6,70	6,64	31,00

following indices were used for oral health status evaluation: DMFT (Decayed, Missing and Filled Teeth) for caries experience, DI-OHI (Debris Index – Oral Hygiene Index) for oral hygiene status, PMA (Papilla, Marginal gingiva, Attached gingival inflammation), CPITN (Community Periodontal Index and Treatment Need) for periodontal status and RI (Restorative Index) for restorative treatment need assessment. A salivary screening test for AIDS* was used. If the patients were restricted to the hospital an ART (Atraumatic Restorative Treatment) was conducted. This treatment was accompanied by a questionnaire, which mapped the type of drug, duration of addiction and attitudes of the addicts towards dental care.

Data processing

The end data were processed using standard statistical tools: means, standard deviations, variance and Student t- test. The results in percentages express frequency of persons according to particular CPITN score.

RESULTS

For the purpose of statistical analysis, the patients were divided into two groups - drug addicts and alcohol addicts. In this paper, only the data on oral health of drug addicts are presented with special reference to the DMFT and CPITN indices. The number of drug-addicted persons was 217 and Table 1 shows caries experience and dental health status of individual age groups with different average duration of addiction. In the age group 15-19 yrs (43 persons), a mean DMFT value of 6.51 per person was found while average duration of addiction was 4.71 years. In the age group of 20-24 yrs (104 persons) a mean DMFT of 8.93 per person was found while average addiction was 6.21 years. In the last group of 70 persons (subjects older than 25 yrs), the mean DMFT value was 12.79 with an average addiction of 6.64 years. It is obvious from the data in Table 1 that the major proportion of DMFT values is formed by decayed and missing teeth.

The CPITN periodontal index observations are presented in Table 2. CPITN score of 0 (healthy periodontal tissues) was found in 8.76 % of subjects, CPITN 1 (bleeding on probing) was found in 8.76 % of examined persons, calculus (i.e. CPITN score 2) in 72.35 %, shallow pockets were found in 4.15 % and deep pockets in 0.92 %. Non-measurable sextants were found in 5.07 % of subjects.

DISCUSSION

The oral health status of drug and alcohol addicts has been discussed and studied extensively in countries that have faced the problem of drug addiction for many years. The dental health of drug addicts has been described in Italy,[3] in the Netherlands[4] and in Denmark[5] and the attitudes of drug addicts towards dental care were reported by Sainsbury in Australia[6] and Scheutz in Denmark.[7] In the Czech Republic, on the other hand, very little attention has been paid to the issue of drug addiction[8-10] because the problem has only recently become

Table 2: *CPITN values of individual age groups of examined patients.*

CPITN score	Number of persons	Respective percentage proportions
0	19	8.76
1	19	8.76
2	157	72.35
3	9	4.15
4	2	0.92
x	11	5.07

* *SDS International Company*

Fig.1: 23 year old male

Fig.2: 28 year old woman

severe. The aspect of our project described in this paper contains information on oral health of drug addicts reflected in the two most relevant indices, DMFT and CPITN, commonly used in epidemiological studies. While the results shown in the tables represent the mean values or percentage proportions of patients with different stages of periodontal disease, the case reports reflect the fact that the patient, for example, who is epidemiologically characterized with DMFT 5, which could be quite favourable (e.g. five fillings), is in reality more likely to have 5 apical abscesses or root remnants and may include atypical semi-lunar caries typical of drug addicts.[11] Similarly, percentage proportions of persons with particular periodontal impairment do not reflect a real picture of what a dentist can encounter in the surgery.

Two cases of oral health status of drug addicts follow and in both cases the state of the dental or periodontal tissues do not correspond with the age of the patient.

Case reports

Case 1

The oral health status of a 23 year-old man is shown in Fig. 1. There has been abuse of alcohol and cannabis from the age of 14 yrs and heroin since age of 17 yrs, and difficulties related to heroin misuse were the reason why he started withdrawal treatment. In the upper jaw the atypical semi-lunar caries was present on the teeth 13, 12, 11, 21 and 22, while tooth 23 was fractured due to the progress of the caries in totally encircling the tooth. Similarly, in the lower jaw this type of caries was found on teeth 34, 33, 43 and 44, the DMFT value was 25 and the gingival margins were inflamed (CPITN of 1-2).

Case 2

Fig. 2 presents the oral health status of a 28 year-old woman addicted to cannabis since the age of 18 yrs and to heroin since age of 22 yrs. The remnants of the dentition are decayed, the upper jaw was edentulous and in the lower jaw the roots of teeth 44 and 43 and the decayed remnants of teeth 31, 41 and 42 remain. The DMFT value was 27, the teeth are covered with plaque due to the lack of oral hygiene and the attached gingivae were inflamed (CPITN, where the sites could be measured, was 3 and 4).

CONCLUSION

The results of the survey indicate that patients being treated for drug addiction in long-term hospitals should undergo dental treatment in order to reduce inflammation and pain and to prevent further destruction of teeth and periodontal tissues in order to restore masticatory function. It is unacceptable to delay dental treatment until the patient leaves the hospital as withdrawal treatment usually takes several months or a year or even longer. The authors suggest that whenever an extreme case of destruction of dental and periodontal tissues or atypical semi-lunar caries is found in young patients the cause may be drug addition. It is also suggested that treatment in a private practice, or examination of a dead body during autopsy be focussed to take into consideration the nature of the case. When examining a dead body, the pattern of oral disease can be indicative of a factor within the history or even in the cause of death.

ACKNOWLEDGEMENT

The Internal Grant Agency of the Ministry of Health of the Czech Republic No.NK/6246-3 is acknowledged for the financial support of this study.

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DIGITAL RADIOGRAPHY AND ELECTRONIC DATA STORAGE FROM THE PERSPECTIVE OF LEGAL REQUIREMENTS FOR RECORD KEEPING

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ABSTRACT

In some countries physicians and dentists are required by law to keep medical and dental records. These records not only serve as personal notes and memory aids but have to be in accordance with the necessary standard of care and may be used as evidence in litigation. Inadequate, incomplete or even missing records can lead to reversal of the burden of proof, resulting in a dramatically reduced chance of successful defence in litigation. The introduction of digital radiography and electronic data storage presents a new problem with respect to legal evidence, since digital data can easily be manipulated and industry is now required to provide adequate measures to prevent manipulations and forgery. **(J Forensic Odontostomatol 2003;21:40-4)**

Keywords: Duty to keep records, digital radiography, electronic data storage, burden of proof

In some countries physicians and dentists are required by law to keep medical and dental records. Together with the duty to take due care and the duty to provide information, this requirement constitutes one of the most significant responsibilities towards the patient.[1,2,3] The practice of record keeping regarding to patient treatment is certainly as old as the practice of medicine itself and is a vital aspect of coherent, coordinated and cogent diagnostics and therapy. What have, however, undergone a fundamental change in the course of time are the legal classification, function and character of medical records. Whereas they were once seen largely as an informal "aide memoire" for the doctor, to ensure coherence and continuity of a treatment, a precedent ruling by the 6th division of the German Federal Court of Justice in 1978 [4] brought about a radical change in prevailing opinion on the legal status of mandatory record-keeping. Records, not only adequate for medical practice, but which left the individual doctor plenty of subjective leeway, ceased to be normal procedure. The new ruling by the Federal Court of Justice stated that the doctor had to make the necessary recordings on the findings and diagnoses made and on the measures and therapies implemented while carrying out his profession. It was thus mandated that the objective measure of the medical duty to take due care, deriving from § 276 of the German Civil Code, i.e. the objectively necessary care, [5] was now to apply to medical record-keeping too. Primarily, it is no longer what the individual doctor considers subjectively as adequate for records but rather what is objectively seen as being essential to meticulous record keeping that now becomes a legal requirement.

This precedent ruling was expressed in the regulations governing the medical and dental professions, which are largely consistent in their wording and spirit: "The necessary records are to be kept on the findings made and the measures taken while carrying out professional activities. Medical records are not merely reminders; they also serve the interests of patients in proper record-keeping."

According to case law and to the vocational regulations adapted to it, then, the physician and the dentist are contractually accountable to the patient with respect to what they have diagnosed and what measures they have taken.

The legal aspect of the duty to keep records develops its impact in medical liability litigation, especially in evidentiary law. Medical failure litigation is characterized by a specific lack of evidence provided by both parties, but especially by the patient. To illustrate this, some evidentiary principles of the law of civil procedure are outlined in brief.

Each party bears the burden of proof for the presence of the actual preconditions of the legal standard in its favour. If, then, one party asserts a claim for compensation, it is within its obligation to present those facts which are essential for the existence of such a claim according to civil law in order to convince the court. The burden of proof gains practical significance if the issue from which one party wishes to derive a claim, or individual facts relevant to the decision, cannot be verified even when all reasonable and available means have been exhausted (referred to as "non liquet", i.e. lack of clarity).

In those cases, the lack of proof will be detrimental to the party bearing the burden of proof for the respective fact. If, then, a "non liquet" remains in the lawsuit, the party bearing the burden of proof will lose the case. If, accordingly, a patient goes to law because they were or believed to have been treated defectively, they bear on principle the burden of proving in their submission that their physician or dentist had caused impairment negligently and culpably to them during the treatment. In most cases, however, the patient, as a medical novice, initially has substantial problems in furnishing this proof because of lack of medical knowledge. In many cases the issue can only be cleared up by accessing the medical records. If these records are unavailable or incomplete, the patient will find themselves in a hopeless situation because of their inability to prove their submission in the event of the categorical rule of the burden of proof being applied consistently. This would obviously be in crass contradiction of the supremacy of the rule of law and to the constitutionally guaranteed equality of the litigating parties. In such cases, the furnishing of proof by the patient was facilitated by giving the judges greater scope in interpreting this law. Such facilitation can go so far as to reverse the burden of proof completely if one party culpably makes the furnishing of proof more difficult or even impossible for the other. This applies in particular if a physician or dentist infringes their duty to keep medical or dental records, i.e. fails to take necessary notes, makes notes belatedly or even alters notes.

At court, the physician or dentist has to explain their treatment procedures to the patient instituting the legal proceedings. This requirement is fulfilled by submitting properly kept records. If the dentist or physician fails to submit appropriate, and adequate records, the "non liquet" in the legal dispute is imposed on them. The term "principle of the equality of litigious weapons" was introduced in this context. [6]

Another important advance in evidentiary law in favour of patients was made by the German Federal Court of Justice in 1982. With several lower-instance courts having affirmed the patient's right to inspect their records,[7,8] the Federal Court of Justice as the supreme court categorically gave the patient pre- and extra-trial access to these records. [9,10,11]

The fundamental right of the patient to inspect their records and to have them surrendered to them was confirmed and substantiated by a number of subsequent judicial decisions. According to the Federal Court of Justice, the physician or dentist is not allowed to oppose the serious wish on the part of the patient to view the notes of their health status and treatment administered. This additional contractual claim was derived from the patient's fundamental right to self-determination and personal dignity. [12]

This raises the additional question of what is covered by the duty to keep records, in particular whether radiographs are also classified as part of these records. Radiographs are not records in the narrower sense, but a verdict delivered by a German Higher Regional Court clearly illustrates that the principles underlying the duty to keep records are also applied to radiographs in court decisions. In the case adjudicated by the Higher Regional Court, [13] a female patient had had two dental implants inserted in the left and right lower canine regions. The implants had to be removed some time later because of fracture, loosening and inflammation. The patient sued the dentist for compensation and damages for personal suffering, asserting that the implants had not been placed in accordance with accepted proce-

dures. The Higher Regional Court explained that it is a principle that the patient has the burden of presentation and proof for all aspects underlying the claim. Under certain circumstances, however, the patient can benefit from facilitation that may go even as far as reversal of the burden of proof. [14] Consideration must be given to such a reversal if circumstances for which the physician or dentist is responsible have given rise to special impediments to clarification of the causes of the reported complications. This might be the case in the event of a gross treatment error, e.g. a fundamentally incorrect diagnosis, or of failure to perform control examinations, if the physician or dentist had failed to perform examinations and to record the findings, even though this was medically necessary beyond all reasonable doubt, or if incomplete records resulted in there being no opportunity to determine whether necessary measures were in fact taken.

Because there were no postoperative radiographs of the implant sites, the principles underlying the probative burden were applied to the detriment of the dentist. It could no longer be assessed whether the complications had been due to the implants having been incorrectly inserted or to inflammatory processes beyond the dentist's control. In such a case, postoperative radiographs are essential not only as evidence but also as a means of preventing harm to the patient. As faulty implantation is just as likely a cause of failure as any other factor, the dentist had to bear the consequences of being unable to clarify the reasons for failure.

This example shows that court decisions subject radiographs to the principles of the duty to keep medical records. Beyond their diagnostic and therapeutic purpose, radiographs have, of course, an exceptionally high documentary value.

In recent times, the duty to keep medical and dental records and the legal problems inherent in this duty have taken on a new aspect with the increasing relevance of computer-aided documentation and digital radiographs. Electronically and digitally recorded radiographs and other data tend to be problematic from the judicial point of view because they can be manipulated. Manipulating conventional radiographs involves relatively high technical effort, whereas digital data can be modified with relative ease. This accounts for the problems involved in classifying digital radiographs as legally evidentiary records. [15]

Let us return to the case at the Higher Regional Court, where the burden of proof was facilitated for the patient because postoperative radiographs had not been taken. Had such radiographs been taken and had they shown the implants to have been inserted perfectly, the dentist would not have been made liable. On the other hand, the possibility of a postoperative digital radiograph having originally shown the implants to have been imperfectly inserted but having been manipulated – without the patient being aware of this – so as to represent normal findings illustrates the problems of digital radiographs being submitted as evidence. [16, 17]

This problem is very much the same with all digital medical records, [18] which – at least until now - have not been accorded the same authenticity as traditional, handwritten records because they can be readily manipulated. The latter can, of course, also be manipulated, but there seem to be greater scruples about doing this because the actions involved in forgery - such as erasing, overwriting and changing - are more likely to activate a sense of guilt than adapting digitally recorded data, which might be perceived as on par with correcting typing errors or merely exploiting modern technology. Moreover, there is a greater likelihood of such manipulations being detected in conventional rather than in digital records.

The criminal energy invested in manipulating a digital radiograph is probably equal to that involved in forging a hand-written record.

Nevertheless, there is a general consensus in the legal literature and in courts that digital radiographs and other digital data need protection from unauthorized manipulation and must be made secure. In the case of image data files, conservation of the unprocessed original records is essential and subsequent manipulations such as rotation, modification of gradation and brightness, correction of gamma values etc. must be saved to serve as a complete track record and evidence of the manipulation.

Another legal question arises from the risks concerning data security, especially the long-term availability of the image data and details of the imaging process and its circumstances. In addition to protecting personal data from unauthorized access and transfer to third parties, digital radiography requires an additional safety feature: protection against inadvertent loss. Twenty eight of the German Radiography Ordinance regulates the obligation to keep radiographs and notes on radiographs. It stipulates *inter alia* that radiographs have to be kept for at least ten years and thus stipulates indirectly that all technical systems required for data access have to be maintained for this period. This implies an exceptionally long-term technical and therefore economic dependence of the dental practice on the manufacturer of the digital radiography unit.

In addition to the Radiography Ordinance, the regulations governing the safekeeping period for medical and dental records mostly specify ten years unless a longer period is stipulated.

In addition, these regulations require special security and protective measures for digital recordings on electronic data carriers or other storage media to prevent them from being manipulated, irreparably damaged or used for unauthorized purposes.

Digital radiography has so many advantages over conventional techniques that banning its use in consideration of legal uncertainties would seem to be unrealistic. Manufacturers and users of digital radiography and computer-based documentation systems must bear in mind not only the organisational and economic aspects but also the value of the records as legal evidence. This can simply be solved by devising security measures, which have in fact already been implemented and involve only moderate expenditure on software supplementation, e.g. digital signature technology (DST). [19,20,21] Security against unauthorized access to digital records also has to be guaranteed.[22] It will probably take some time for these new systems to become widely used but there is no doubt that electronic data acquisition systems are here to stay and are an innovative fact of life.[23]

Apart from their fundamental importance, including dental identification, [24] dental records are also gaining steadily in importance as legal documents. Many patients will, once a lawsuit is under way, be seized by tactical amnesia and challenge everything that has gone before. In written briefs this is worded roughly as follows: All statements by the opposing party are disputed unless otherwise explicitly acknowledged. In such situations, well kept records are of inestimable value to the dentist, especially if they carry the probative burden (e.g. for correct informing of the patient and for the patient's consent to treatment), especially as the jurisdiction makes high demands on the quality of medical and dental records while taking the view that adequate records are plausible in general.[25] And yet, many liability cases expose the whole dilemma of inadequate or even missing documentation, inevitably leading to a deterioration in the dentist's litigious position.

As a conclusion, it can be stated that there is reason enough for the dentist or physician to pay attention not only to their duty to take due care and to provide the patient with adequate information but also to their record-keeping duty, to avoid being caught with their back to the wall. This is true for conventional as well as electronic records and radiographs

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