ISSN 2219-6749

I·O·F·O·S

The Journal of Forensic Odonto-Stomatology

Volume 15, n. 1 - Jun 1997

OFFICIAL PUBLICATION OF THE
INTERNATIONAL ORGANIZATION FOR
FORENSIC ODONTO-STOMATOLOGY

CONTENTS

A PILOT STUDY TO DETERMINE THE ORDER OF EMERGENCE OF PERMANENT CENTRAL INCISORS AND PERMANENT FIRST MOLARS OF CHILDREN IN THE COLCHESTER AREA OF THE U.K.

A. Elmes and E. Dykes
Civil Emergency Management Centre, University of Hertfordshire, College Lane, Hatfield, Hertfordshire, U.K.

ABSTRACT

Over the period of one calendar month, 505 children in the Colchester area of England were examined. The data gathered from the 12 non-Caucasian children was not used, leaving 493 Caucasian children (255 boys and 238 girls). Their mean age was 6.48 years, with a range of 4.49-8.75 years and 164 children had no permanent teeth leaving 329 children with a total of 2,238 permanent teeth present. A permanent tooth was deemed to have emerged if any part of it was visible in the mouth. The forensic significance of the results of this pilot study are that there is no significant difference ($t=0.407$, $P>0.2$) between the age of emergence of permanent central incisors (mean age 6.23 years) and permanent first molars (mean age 6.29 years). On the whole, lower central incisors emerge before upper central incisors and there is no significant difference ($t=0.899$, $P>0.2$) in the emergence times of upper and lower permanent first molars. There is no significant difference ($t=0.876$, $P>0.2$) in the emergence times of permanent central incisors for boys and girls (6.22 and 6.25 years respectively) and there is no significant difference ($t=1.413$, $P>0.1$) in the emergence times of permanent first molars for boys and girls (6.40 and 6.08 years respectively). **(J Forensic Odontostomatol 1997; 15: 1-4)**

Keywords: emergence, permanent first molars, permanent central incisors, forensic odontology

INTRODUCTION

For a multitude of reasons, medical and dental professionals are asked to assess the age of a living child or adult, body, jaw or tooth. They may also be asked to assess biological as compared with chronological age, skeletal, humoral, mental or dental age of an individual. The assessment is undertaken by means of visual, oral, documentary, clinical, laboratory and radiographic techniques.

All the systems in current use are based on a single principle. A large number of 'normal' individuals of known age are examined and their data recorded. Tables are produced which summarise the data and mean ages and age ranges are presented. The details of an unknown individual are then compared with the known population's data and an estimated age can be given. Ideally, more than one method of age assessment is used to reach a final statement regarding the age of an individual.

When a dental age assessment is undertaken several methods may be employed. Some of these are visual, histological, radiographic, degenerative and chemical.

Visual age assessments compare the teeth present in the mouth with standard data of emergence times,[1-5] or with a developmental atlas.[6,7] Radiographic age assessments examine the development, emergence and root formation of deciduous and permanent teeth. Calcification dates, crown completion dates, root formation dates and apical closure dates are used to provide an age assessment.[8-10]

Once a person is over the age of 21 or 22 years, when third permanent molars have completed their development, it is necessary to examine the changes in the dentition to estimate age. Gustafson[11] produced the most widely known system for assessing age by degenerative changes of teeth. He based his system on attrition, periodontosis, secondary dentine deposition, cementum apposition, root and root translucency. Others have modified and simplified his system.[12-14]

Over the years many ions and organic molecules have been investigated to see if their concentrations give an indication of age. Most recently D-aspartic acid in dental collagen has been studied as an age indicator,[15] but so far no one chemical has been found as the ideal marked for dental age assessment.

When systems for age assessment depend on deciding whether, for example, a quarter of the crown or half of the root is mineralised, they are dependent upon the subjective observation of an expert assessor. On the other hand specialist experience is not needed to say whether a tooth is present or absent, so in this pilot study this criterion was used as the deciding factor. This system has the advantage that when no radiographic or other specialist equipment is available, for example at the site of a mass disaster, it can be applied simply and accurately.

Once tables of mean emergence dates are compiled, whichever system is used, an emergence sequence can be drawn up. Most standard dental and forensic odontological texts tend to quote the work of Schour and Massler.[6] Thus for the permanent dentition, the permanent first molars emerge first, between six and seven years of age followed by the lower permanent central incisors between the ages of six and eight. The upper permanent central incisors are the third in the sequence, emerging between the ages of seven and nine. This is shown pictorially in the American Dental Association charts, currently reproduced by Ciapparelli.[16]

The aim of this study was to verify this forensically accepted emergence sequence given the fact that it is not always clinically observed. This may be important when dental age assessments are used in the identification of children in the age group under consideration.

METHODS AND MATERIALS

With the cooperation of a local Community Dental Officer, six primary schools in the Colchester area were visited over a period of one month and 505 children's mouths were examined whilst they were sitting in an ordinary chair with adequate lighting using a mirror and probe for each child. While the School's Dental Examination was undertaken, the permanent teeth present in the mouth were also recorded as were the school, the date of examination, gender, date of birth and racial group.

In this study, a tooth was classified as present as soon as it pierced the gingiva. It didn't matter if this had only just happened, if the tooth was half-way to the occlusal table or if it had reached the occlusal table. All permanent teeth which were present in the oral cavity were deemed to have emerged. All teeth which were not present, whether because of non-formation, non-emergence or extraction were deemed to be absent. Statistical analyses were conducted using an unpaired (variances equal) t-Test.

RESULTS

All but 12 of the 505 children examined were of Caucasian origin (the 12 being of mixed race) and the mean age was 6.46 years, range 4.49 - 8.75 years. There were 255 boys and 238 girls, having a total of 2,238 permanent teeth present (boys 1,061 - girls 1,177). In 164 children no permanent teeth had emerged and these consisted of 89 boys (mean age 5.59 years, range 4.49 - 7.22 years) and 75 girls (mean age 5.50 years, range 4.86 - 6.49 years).

The remaining 329 subjects with permanent teeth (Table 1) had a total of 870 permanent central incisors, 1049 permanent first molars, 314 permanent lateral incisors and five permanent canines. The numbers of permanent lateral incisors and permanent canines were too small to analyse statistically.

Tooth (FDI)	Boys n=166	Girls n=163
11	71	88
21	70	84
31	135	144
41	137	141
12	19	29
22	17	28
32	48	62
42	47	64
13	0	0
23	0	0
33	0	1
43	0	4
16	129	133
26	125	131
36	133	134
46	130	134

Table 1. Distribution of Permanent teeth in the 329 Children

Of the total number 205 of the children had permanent central incisors and/or permanent first molars present (and no other permanent teeth had emerged) at the time of examination (Table 2). As the main aim of this survey was to examine the mean age of emergence of permanent central incisors and permanent first molars separately we only used the data from the 33 children who had only permanent central incisors and no permanent first molars present (Table 3) and 38 children who had only permanent first molars present and no permanent central incisors present (Table 4). For the other 134 children, we had no information as to which of these teeth had emerged first.

DISCUSSION

Table 1 shows that girls consistently had more teeth in each group present in the mouth, than boys. The evidence from all the data presented (Tables 1, 3 and 4) would suggest that there is no predisposition as to which homologous pair of teeth emerges first as in all cases there

	n	Permanent Central Incisors lower	Permanent Central Incisors upper	Permanent First Molars lower	Permanent First Molars upper
Boys	112	164	41	157	146
Girls	93	145	42	130	125
Total	205	309	83	287	271

Table 2. Children with Permanent Central Incisors and/or Permanent First Molars Only.

	n	Mean Age & Range in Years	Tooth 11	Tooth 21	Tooth 31	Tooth 41
Boys	18	6.22 (5.32-7.11)	2	1	16	18
Girls	15	6.25 (5.16-7.72)	0	0	13	13
Total	33	6.23 (5.16-7.72)	2	1	29	31

Table 3. Children with Permanent Central Incisors Only (FDI 11, 21, 31 & 41).

	n	Mean Age & Range in Years	Tooth 16	Tooth 26	Tooth 36	Tooth 46
Boys	25	6.40 (4.91-7.57)	20	16	18	18
Girls	13	6.08 (5.23-7.29)	9	9	8	11
Total	38	6.29 (4.91-7.57)	29	25	26	29

Table 4. Children with Permanent First Molars Only (FDI 16, 26, 36 & 46).

are equivalent numbers of left and right teeth. There are similar numbers of upper (518 out of the maximum of 658 for the 329 children) and lower (531) permanent first molars but many more lower permanent central incisors (557) than upper permanent central incisors (313, see Table 1). The implications from this are that permanent first molars emerge before permanent central incisors and that lower permanent incisors emerge before upper permanent central incisors. However, there is no significant difference ($P>0.2$) when one looks at the dates of emergence for the central incisors alone (mean 6.23 years - Table 3) and the permanent first molars alone (mean 6.29 years - Table 4). This finding is in disagreement with some authors[5] but in agreement with others.[2,3] Lavelle[4] is unhappy to predict sequences of emergence and acknowledges individual variation in genetics, race, socio-economic groups, health, hormones and sampling times.

From Table 3 it can be seen that 60 of the maximum of 66 lower central incisors have emerged whereas only 3 of the maximum number of 66 upper central incisors have emerged indicating that lower central incisors emerge before upper central incisors. There is no corresponding evidence for the same conclusion to be drawn for the permanent first molars (54 out of a maximum of 76 upper permanent first molars emerged and 55 out of 76 lower permanent first molars emerged) (Table 4).

In a study of eruption times[2,3] it was reported that for boys the upper and lower permanent first molars emerge simultaneously but girls' lower permanent first molars emerge first. Our limited survey has found no significant difference between boys and girls and the emergence of permanent first molars. In another study[5] it was observed that permanent first molars and lower permanent central incisors emerge simultaneously followed by upper permanent central incisors. Our results agree with these findings.

CONCLUSIONS

We therefore conclude from this pilot study that there is no significant difference between the ages at emergence of permanent central incisors and permanent first molars. Lower central incisors emerge before upper central incisors and there is no significant difference in the emergence times of upper and lower permanent first molars. There is no significant difference in the emergence times of permanent central incisors and permanent first molars for boys and girls.

Address for correspondence:
Dr E Dykes
Civil Emergency Management Centre
University of Hertfordshire
College Lane
Hatfield
Hertfordshire AL 10 9AB
United Kingdom

REFERENCES

1. Ainsworth NJ. The incidence of dental disease in children. Medical Research Council Special Report Series No.97, 1925.

2. Clements EMB, Davies-Thomas E, Pickett KG. Time of eruption of permanent teeth in British children in 1947-8. BMJ 1953a; 1: 1421-4.

3. Clements EMB, Davies-Thomas E, Pickett KG. Order of eruption of the permanent human dentition. BMJ 1953b; 1: 1425-7.

4. Lavelle CLB. The timing of tooth emergence in four population samples. J Dent 1976; 4: 231-6.

5. Miller J, Hobson P, Gaskill TJ. A serial study of the chronology of exfoliation of deciduous teeth and eruption of permanent teeth. Arch oral Biol 1965; 10: 805-17.

6. Schour I, Massler DDS. The development of the human dentition. J Am Dent Assoc 1941; 28: 1153-60.

7. Van der Linden FPGM, Duterloo HS. Development of the human dentition. An atlas. Baltimore: Harper and Row, 1976.

8. Demirjian A, Goldstein H, Tanner JM. A new system of dental age assessment. Hum Biol 1973; 45: 211-27.

9. Moorrees CFA, Fanning EA, Hunt EE. Age variation of formation stages for ten permanent teeth. J Dent Res 1963; 42: 1490-502.

10. Nortje CJ. The permanent mandibular third molar. Its value in age determination. J Forensic Odontostomatol 1983; 1:27-31.

11. Gustafson G. Age determinations on teeth. J Am Dent Assoc 1950; 41: 45-56.

12. Bang G, Ramm E. Determination of age in humans from root dentine transparency. Acta Odont Scand 1970; 28: 3-35.

13. Lorensten M, Solheim T. Age assessment based on translucent dentine. J Forensic Odontostomatol 1989; 7: 3-9.

14. Solheim T. Dental age estimation. An alternative technique for tooth sectioning. Am J Forensic Med Pathol 1984; 5: 181-4.

15. Gillard RD, Pollard AM, Sutton PA, Whittaker DK. An improved method for age at death determination from measurement of D-Aspartic acid in dental collagen. Archaeometry 1990; 32: 61-70.

16. Ciapparelli L. The chronology of dental development and age assessment. In: Clark DH, ed. Practical Forensic Odontology, Bristol: Wright, 1992: 22-42.

A HIERARCHICAL SYSTEM FOR THE CODING OF DENTAL INFORMATION IN REPORTS AND COMPUTER-ASSISTED IDENTIFICATIONS

T Solheim
Department of Oral Pathology and Section for Forensic Odontology, Faculty of Dentistry, University of Oslo, Norway

ABSTRACT

Dental coding systems have hitherto been developed mainly to record treatment. The present hierarchical system was specially designed for computer aided dental identification and has been tested for 15 years in the Norwegian register of missing persons. It is in addition well suited for rapid and exact reporting of dental findings in identification cases. The system has also been proposed for adoption by Interpol. **(J Forensic Odontostomatol 1997; 15: 5-8)**

Keywords: forensic dentistry, identification, computers, disaster planning

INTRODUCTION

Except for the FDI system for coding dental surfaces and the two digit system for designating teeth, no international standards exist for codes and abbreviations of dental nomenclature. A number of systems based generally on the national language have been developed in different countries[2] and these may satisfy the need for standards in general clinical dentistry. However, few countries have established national standards, though producers of commercial computer programs for administration of dental practices and recording of treatments have developed their own systems.

As the standard of dental recording varies considerably, forensic odontologists often have to work with information from dental practice which is less than exact. Furthermore, the brand name of a restorative material found when examining a dead person will usually be unknown unless special analyses are carried out. These uncertainties may cause problems during the comparison and also when the forensic odontological report is compiled. Traditionally, forensic odontologists have either written the findings in their reports in full or have used their own abbreviations, which may be incomprehensible for their colleagues. Systems have been suggested, often based on numerals[3,4] but have so far been received with little enthusiasm, probably because it is more difficult to work with figures that do not give any associations, and also because computers today can handle text just as well as numbers. The result of this diversity of systems is that the reports from different forensic odontologists differ greatly in appearance, as may be illustrated by a case from Scandinavia.[5]

As long as the reports are not computerized or intended to be used for computer search, these diversities in the description of the dental findings may not be a serious problem for the odontological identification method. The effect may even be positive, as it encourages inventiveness, so that a number of systems are in use, though seldom evaluated.

With the introduction of computer programs for registration of dental conditions of missing persons and for use in identification in mass disasters, a uniform coding system is necessary because only standard expressions and codes can be retrieved. Besides, a compact coding system may considerably reduce the possibility of typing errors.

For international exchange of dental information regarding unknown missing persons and bodies, especially after mass disasters, there is a need for a standardization, because the existing variety of systems is poorly understood by most workers.[5,6] It is in fact often impossible to decipher much of the dental information received from many countries. In addition, the dental information is sometimes given in such an imprecise way that it appears to have originated from an unqualified source. Interpol is aware of the problem, and the system proposed here is meant to redress it and be considered for Interpol use. Indeed, it has been advocated that a common international system would be an advantage for dentistry in general.[2]

THE COMPUTERIZED NORWEGIAN REGISTER OF MISSING PERSONS

After initial tests in 1980[7,8] the computerized Norwegian register of missing persons was put into operation in 1985. It is based on a police main frame computer, first used with the text retrieval system "Nova*Status" but in 1993 this was replaced by the improved "Shift" program. These are programs developed in Norway for official and police use.

The full Interpol form, including D1 and D2 (the dental parts), is entered into the computer and to provide a full computerized description of the teeth in a limited space we constructed a simple system of codes and abbreviations. Over the years experience has brought about major improvements resulting in the system which is used today and presented in this article.

Since 1992 information on dead persons of unknown identity has been entered into the register together with information on a selection of missing and dead persons from other countries, mostly Swedes and Danes, and the register now contains information on more than 500

persons. The register is only accessible from police computers, and entries and search are usually carried out by police personnel, while the dental coding from dental records has so far only been made by a dentist. The intention is that in future a strict use of the codes by all forensic odontologists in Norway will result in uniform reports that can safely be entered straight into the computer by non-dentally qualified personnel.

After coding, all original documents and radiographs are filed by the police so that when a dead person is found the necessary material for identification is readily accessible. When the police computer has come up with a possible identification the forensic odontologist is called on to compare the reports and also the original documents and radiographs, and it is then easy to carry out whatever additional computer search may be necessary.

THE CODING SYSTEM

The system is based upon a limited number of codes for the features most often used in identification and which may be useful in a computer search. The use of only a limited number of codes has also been recommended by others.[3] For additional details a description in full text will be used and as "Shift" is a text retrieval program a full text string may also be searched for.

The coding system is built up in a hierarchical way,[1] going from general to special information. The codes are constructed simply, a single letter generally being employed for most codes, and to avoid ambiguity one code has only one meaning. The importance of a simple coding system has been emphasized by others and some requirements for such a system have been proposed.[9]

The description of a tooth starts with a status for which the codes are given in Table 1. As some types of information in a status may differ from a missing person and from a body whose identity is unknown, the exact interpretation of each code may vary somewhat, but in general will be the same. For example, the code Z for a missing person means that we have no information about a tooth or when a part of the jaw containing a particular tooth is missing from a dead person. In that case we do not even know whether the tooth was present or not, and Z is used.

S	= sound tooth
C	= caries
F	= filled tooth
K	= crown
W	= remaining roots(s) only
X	= tooth missing (extracted, unerupted, congenitally missing)
Y	= tooth present, no further information/tooth lost post-mortem
Z	= no information about the tooth/part of jaw lost post-mortem

Table 1. Dental status codes. The information is based upon the clinical examination, but radiographs may also contribute to this status, especially in a missing person.

One code usually covers the information available for one tooth but it is possible to use more than one status code simultaneously, e.g. a tooth may have a crown (K) and in addition a filling (F) and/or caries (C).

Supplementary codes may give information additional to the status codes, e.g. the type of material used in a filling and the surfaces filled. Such supplementary codes are given in Tables 2-6. For coding of the surfaces the FDI[10] and ISO-recommended nomenclature is used (Table 7).

It is sometimes deemed important to state specifically which part of a dental surface is include, so an additional

ERU	= tooth in eruption
RET	= tooth retained (impacted) but visible in the mouth (partially impacted)

Table 2. Supplementary codes for sound teeth (S). Capital letters are recommended

am	= amalgam
t	= tooth-coloured filling (composite, glassionomer, silicate)
g	= gold
p	= porcelain
ac	= acrylic
ce	= cementum (temporary restoration)
POS	= post (pulpal anchorage)
PIN	= parapulpal pin

Table 3. Supplementary codes for filed teeth (F). Small letters are recommended except for post and pin. The surface should be indicated after the filling material by using M O D L V according to the FDI recommendations.

g	= gold
p	= porcelain
ac	= acrylic
am	= amalgam
t	= tooth-coloured filling (composite, glassionomer, silicate)
ce	= cementum (temporary restoration)
POS	= post (pulpal anchorage)
PIN	= parapulpal pin
B	= bridge (tooth is an abutment in a bridge)

Table 4. Supplementary codes for teeth with crowns (K). Small letters are recommended for the material

POS	= post (pulpal anchorage)
PIN	= parapulpal pin

Table 5. Supplementary codes for remaining roots (W).

U	= diastema. Distance may be added in millimeters e.g. U4 = diastema of 4 mm
RET	= retained (impacted) tooth, only visible on X-rays
ROT	= rot in the jawbone, only visible on X-rays
E	= extension of a bridge to replace the tooth
H	= bridge pontic

Table 6. Supplementary codes for tooth missing or extracted (X).

M = mesial
O = occlusal, incisal
D = distal
L = lingual, palatal
V = vestibular, labial, buccal

Table 7. Supplementary codes for tooth surfaces.

mes = mesial
occ = occlusal, incisal
dis = distal
lin = lingual
ves = vestibular
cen = central
gin = gingival

Table 8. Three-letter codes to designate a part of a surface. Small letters are recommended.

11 F t M D	21 F t M.
12 F am L, t M.	22 Z.
13 S.	23 Z.
14 F am M O D	24 F t M O C D.
15 K pg.	25 F t M O D.
16 F am M O D L.	26 X UO.
17 X.	27 F am M O.
18 Z.	28 X.
48 Y.	38 X RET
47 K g	37 F am M, O, V.
46 X U4	36 F am M, O, V gin.
45 F t M O-mes.	35 K pg POS.
44 S.	34 F t V.
43 Z.	33 Y.
42 Z.	32 Z.
41 Z.	31 Z.
19 D. 29 YES.	39 Z. 49 27 35

Table 9. Example of coding of the dental condition of a missing person

set of abbreviations has been designed for these occasions. To differentiate this part clearly a three-letter code is used and small letters are recommended[11] (Table 8).

An example of a dentition coded with this system is given in Table 9. To be interpreted by the computer as separate words the codes are separated from each other by a space while codes for fillings that are not adjacent to each other may be separated by a comma.

The coding of 19 (occlusion), 29 (smoking habit), 39 (dentures) and 49 (root-filled teeth) at the end is optional, and indeed the Interpol form does not provide for them. These codes have however been found to be useful in the Norwegian register of missing persons and are inserted under no.87 in the Interpol form and are shown in Table 10.

19 OCCLUSION AND DENTAL POSITION
N = Normal occlusal relationship between first molars
D = distal occlusal relationship between first molars
M = mesial occlusal relationship between first molars
CU = crowding in upper jaw (maxilla)
CL = crowding in lower jaw (mandibular)
H = horizontal relation betwen maxillary and mandibular incisors (H4 = maxillary teeth 4 mm anterior to mandibular)
V = vertical relation between incisors
Z = no information
29 SMOKING HABITS
YES/NO (where known)
Z = unknown
39 DENTURES
FU = full upper (maxillary denture)
FL = full lower (mandibular denture)
PU = partial upper (maxillary)
PL = partial lower (mandibular)
CC = chrome-cobalt skeleton
Z =no information about dentures
49 ROOT-FILLED TEETH
Use tooth number to indicate which teeth are filled
Z = no information about root-filled teeth

Table 10. Specific coding of fields no 19, 29, 39 and 49.

Green = tooth-coloured material (composite, silicate, resin, glass ionomer and cementum)
Blue = amalgam and amalgam-coloured materials
Red = gold
Black = other materials such as casts of non-precious metals

Table 11. Colour codes for use in the odontogram

EXPERIENCE

The author has used these codes during the past 10 years for reporting in all cases of examination of deceased persons and in systematizing information on missing persons. Experience is that this is a rapid and exact way of describing the dentition, that additional descriptions are seldom needed, that mistakes are rare, and above all, that with training, the method gives almost as good an overview of the condition of the teeth as drawing the fillings onto the odontogram. However, the odontogram is usually filled in in any case, as it may in some respects supplement the coded description.

Norwegian forensic odontologists have not as yet been required to use these codes. Implementation of any new system takes time and this country's reports are thus not uniform. With the emergence of the requirement for quality assurance in identification work, this is one of the factors that will have to be standardized.

The codes have been strictly used on all missing person reports to be entered into the police computer. Police secretaries do the computerization and scarcely any mistakes have been detected. Furthermore, it is important for the secretaries to be able to use the dental information for searching purposes, and in discussions with the forensic odontologist to understand the meaning of the codes. In my view this is a great advantage when working with police officers, even though they usually have no knowledge of the codes.

In all cases where the police believe they have a possible identification, the forensic odontologist has been consulted to examine the original report, records and radiographs in order to make the final identification.

COLOUR CODES

Colour codes indicated in Table 11 are used for drawing the details of the fillings and crowns onto the odontogram.

Address for correspondence:
Tore Solheim
Department of Oral Pathology and Section for Forensic Odontology
Box 1109 Blindern
N-0317 Oslo
Norway

REFERENCES

1. Date CJ. An introduction to database systems. 6th ed. Addison-Wesley: Reading, Massachusetts, 1995: 22-3.

2. Kimmel K. Code system für zahnärztliche Massnahmen. Quintessenz 1970; 21: 131-2.

3. Keiser-Nielsen S. A six-digit code for computer aided dental identification. Forensic Sci Int 1983; 21: 85-9.

4. Villa Vigil MA, Arenal AA, Rodriguez Gonzalez MA, Llompart RC, Gonzalez Menendez JM, Diez FR. Simplified method of odontograms for individual identification. Quintessence Int 1990; 21: 1013-8.

5. Kullman L, Solheim T, Grundin R, Teivens A. Computer registration of missing persons. A case of Scandinavian cooperation in identification of an unknown male skeleton. Forensic Sci Int 1993; 60: 15-22.

6. Keiser-Nielsen S. Digitilization of dental recording. Forensic Sci Int 1982; 20: 153-61.

7. Solheim T, Rønning S, Hars B, Sundnes PK. A new system for computer aided dental identification in mass disasters. Forensic Sci Int 1982; 20: 127-31.

8. Dahl JE, Solheim T. Computer aided dental identification: Experience from the oil rig "Alexander L. Kielland" disaster. J Forensic Odontostomatol 1985; 3: 7-10.

9. Drum W. Standardisierung der Zahnbezeichnungen. Quintessenz 1970; 21: 155-7.

10. FDI. Marking of dentures and mounting of radiographs. Newsletter 1973; (April): 5.

11. Keiser-Nielsen S. Person identification by means of the teeth. Wright: Bristol, 1980; 1-114.

AGE ESTIMATION FROM RACEMIZATION RATE USING HEATED TEETH

S Ohtani, Y Yamada, I Yamamoto
Department of Forensic Dental Medicine, Kanagawa Dental College, Yokosuka 238, Japan

ABSTRACT

The racemization ratio (D/L ratio) of aspartic acid was determined in soluble peptide and total amino acid extracted from the dentine of experimentally-heated and unheated teeth. The ratio was higher in heated than in unheated teeth for total amino acid, but approximately the same for soluble peptide. Although the estimated age for total amino acid was much greater than the true age for heated teeth, it was approximately the same for total amino acid in unheated teeth and for soluble peptide in heated teeth and unheated teeth. These results suggest that age calculated from the D/L ratio of dentinal soluble peptide from burned bodies is sufficiently accurate for this parameter to be used to estimate age in teeth from burned bodies. **(J Forensic Odontostomatol 1997; 15: 9-12)**

Keywords: forensic science, estimation of age, heated teeth, racemization, aspartic acid, soluble peptide

INTRODUCTION

L-amino acids which comprise the proteins of living tissue are converted into tissue by slow metabolism, or into D-amino acids over a long period, so that the mixture approaches a racemate.[1,2] Using the racemization ratio (D/L ratio) of aspartic acid in total amino acid, the age of teeth from unidentified bodies has been successfully established.[3] In human dentine in particular, D-aspartic acid increases almost linearly with ageing,[4-12] probably because the dentine is surrounded by enamel and cementum and the environmental temperature and water content of the dentinal tubules are maintained at constant levels.[4]

Other studies[6] have reported that the ratio for soluble peptide was highly correlated with age, as was that for total amino acid, when total amino acid was differentiated from insoluble collagen and soluble peptide. Furthermore, it was found that soluble peptide from viable tissue reacts much more rapidly than total amino acid.

Teeth from burned bodies are preserved relatively well in many cases, even when the skin is charred. However, the age of the body of a burned person may occasionally be over-estimated[13] because racemization is accelerated by heating. The reaction rates obtained in experimental heating showed that the pattern of the primary reaction of total amino acid insoluble collagen disappeared with time whereas soluble peptide showed a stable increase in the D/L ratio that reflected the pattern of the primary reaction for a considerably longer time (at least 2h at 180°C).[5] It has also been found that the reaction rate for insoluble collagen was fastest and that the rate for soluble peptide was the slowest upon experimental heating.[5]

These changes in soluble peptide are very convenient for estimating age from the D/L ratio in teeth from burned bodies. To test the reliability of the estimation, the D/L ratio of aspartic acid was determined for total amino acid and for soluble peptide of dentine, comparing teeth which had been experimentally heated with unheated teeth.

MATERIALS AND METHODS

A total of 22 teeth were used in this study. All the teeth used had been extracted because of periodontal disease or for orthodontic treatment. They were kept dry and untreated (for between 3 months and 9 years) and only teeth free of caries invading the dentine were used. Some reports have indicated that racemization does not proceed for long when teeth are kept dry.[4,14]

During preliminary experiments to determine correct concentration of HCl to extract soluble peptide from dentine, powdered dentine (350mg from 10 lower central incisors of 10 persons, aged 42 to 64 years) kept in a desiccator at room temperature was used. To prepare the dentine powder, longitudinal sections of the whole tooth were made with an Isomet saw (type: 11-1180)*.

Enamel and cementum were cut off from the longitudinal sections based on the absence of dentinal tubules with the aid of a stereo microscope. Sections were then washed, pulverized in a Menault mortar** and dried in accordance with the established method.[7] Only whole dentine was used because it has been demonstrated that the coefficient of correlation is better using whole dentine rather than part of it.[11] To prepare the heated specimens the samples were placed in test tubes and heated at 150°C for 1h using an aluminium heating block (type: HF-21)†.

Previous reports have indicated that these were the most ideal conditions to cause racemization as a primary reaction. For the determination of the D/L ratio in total amino acid, 10mg powdered specimens from the 350mg were used. Soluble peptide was extracted by adding 1ml each of 0.5M, 0.8M and 1.0M HCl to each 40mg from the 350mg of the powdered specimen, and by cooling centrifugation (4000 × g, for 1h) at 5°C. The supernatant was then dried with a rotary evaporator. The D/L ratio of

* Buehler, Chicago, USA
** Fritsch, Idav-oberstein, Germany
† Yamato Inc., Tokyo, Japan

aspartic acid was determined[4,6] by gas chromatography using a 25 metre long, 0.3mm internal diameter glass capillary column coated with Chirasil-Val††.

The materials used to estimate age were 12 lower central incisors from 12 persons of known age. Six of the teeth were heated and then reduced to powder, the remaining 6 teeth were not heated. For total amino acid, 10mg from each tooth was sampled and 40mg from the same tooth was used for extraction. From the preliminary experiments, D/L ratios among three different HCl concentrations were almost the same. Therefore, the powdered specimens for soluble peptide were extracted in 1.0M HCl solution. The D/L ratio for total amino acid and soluble peptide were then determined as described above.

RESULTS

Table 1 shows that in the pooled dentine the D/L ratio for aspartic acid in total amino acid was higher in the heated than in the unheated teeth, whereas the ratio for soluble peptide between heated and unheated samples was approximately the same.

Teeth	Total amino acid	Soluble peptide extracted in: 0.5M	0.8M	1.09M
Unheated	0.0982	0.2524	0.2548	0.2554
Heated	0.1676	0.2528	0.2552	0.2546

Table 1. Comparison of D/L ratio for total amino acid and soluble peptide from heated and unheated teeth

The general amino acid racemization reaction can be written as:

$$\text{L-aspartic acid} \overset{k_L}{\rightleftharpoons} \text{D-aspartic acid} \qquad (1)$$

where k is the first-order rate constant of the interconversion of the L- and D-aspartic acid enantiomers. The first-order rate constant for racemization equals 2k. The rate expression is given as follows:

$$\frac{d\,(\text{L-aspartic acid})}{dt} = k\,(\text{L-amino acid}) - k(\text{D-amino acid}) \qquad (2)$$

Consequently, the integrated form of the equation (2) is:

$$\ln[(1+D/L)/(1-D/L)]t = \ln[(1+D/L)/(1-D/L)]_{t=0} + 2kt \qquad (3)$$

where t is any given time (age) during racemization and the logarithmic term at t=0 results from the amounts of D-aspartic acid.

Linear line of racemization can be constructed by plotting the first term, $\ln[(1+D/L)/(1-D/L)]$, of the equation (3) against time (t).[5,15]

†† Applied Science,Inc., Stanford, USA

A plot of $\ln[(1+D/L)/(1-D/L)]$ of aspartic acid from total amino acid (unheated teeth) against the ages of teeth is shown in Fig.1. The line obtained by the linear regression method was as follows:

$$\ln[(1+D/L)/(1-D/L)] = 0.0373+0.0061166t \qquad (4)$$

where t is the age of the tooth. The correlation coefficient (r) was 0.992 and $k=5.8300 \times 10^{-4}$.

Similarly, the case of soluble peptide (unheated teeth) was as follows:

$$\ln[(1\_D/L)/(1-D/L)] = 0.0285+0.004172t \qquad (5)$$

$r=0.997$, $k=2.0860 \times 10^{-3}$

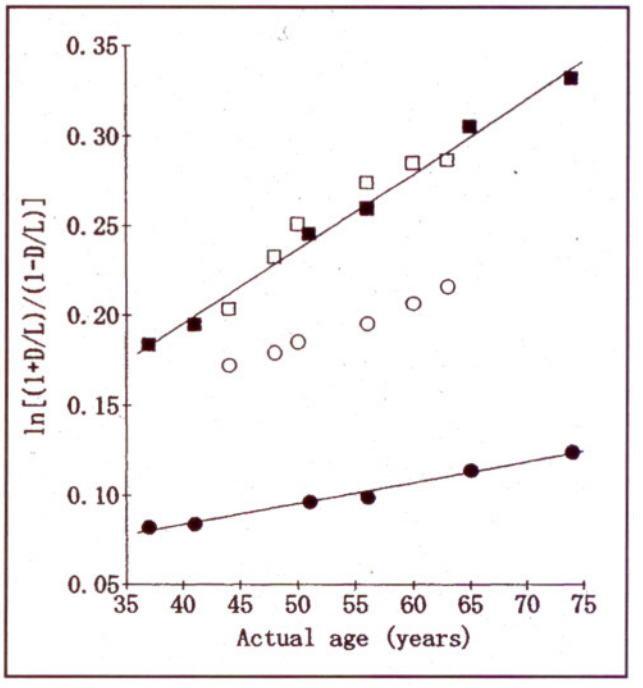

Figure 1. Estimation of age from the dentine by racemization of aspartic acid.
● = *total amino acid, unheated teeth*
○ = *total amino acid, heated teeth*
■ = *soluble peptide, unheated teeth*
□ = *soluble peptide, heated teeth*

Table 2 and Fig.1 show the estimation of age, using the racemization ratio, in six heated and six unheated teeth from persons of known age.

The results have indicated that the estimated age was roughly accurate for both total amino acid and soluble peptide calculations in unheated teeth. In heated teeth it was also accurate for soluble peptide but considerably greater than the actual age when calculated from total amino acid. The rate of racemization of soluble peptide was faster than that of total amino acid (Fig.1).

Teeth	Actual age	Total amino acid D/L	Estimated age	error	Soluble peptide D/L	Estimated age	Error
	37	0.0826	39	+2	0.1838	37	±0
	41	0.0842	40	-1	0.1950	39	-2
Unheated	51	0.0966	50	-1	0.2458	52	+1
teeth	56	0.0994	53	-3	0.2600	55	-1
	65	0.1144	65	±0	0.3058	66	+1
	74	0.1244	74	±0	0.3326	72	-2
	44	0.1726	115	+71	0.2038	42	-2
	48	0.1794	120	+72	0.2330	49	+1
Heated	50	0.1856	126	+76	0.2512	53	+3
teeth	56	0.1958	134	+78	0.2746	58	+2
	60	0.2072	144	+84	0.2856	61	+1
	63	0.2168	152	+89	0.2872	61	-2

Table 2. Estimation of age from dentine by racemization of aspartic acid

DISCUSSION

The D/L ratio for aspartic acid in total amino acid in powdered specimens was higher in the heated than in the unheated teeth. Since racemization is a chemical reaction, the kinetic energy of the molecules is increased by the rise in environmental temperature due to the reaction, leading to an increase in the reaction rate. However, the D/L ratio for soluble peptide from unheated teeth was approximately the same as for heated teeth. The reaction rate of soluble peptide is fast in viable tissue and is decreased by heating,[5] probably because the water content of the dentine decreases. Furthermore, the amount of amino acid converted from the D type to the L type is also increased, thereby causing a decrease in the rate, since racemization is a reversible primary reaction.[15] Soluble peptide was extracted with 0.5, 0.8 and 1.0M HCl, and the D/L ratios ranged from 0.2524 to 0.2554. When calculating age from the formula (5), the difference was ±1 year (53 years and 54 years), the difference in values being very small.

Soluble peptide was extracted with 1M HCl because the amount extracted was large and the results obtained using this method have been shown to be closely correlated with age.[16] The rate of racemization for soluble peptide is about 3.6 times faster than that for total amino acid, and this is believed to be due to the influence of metal ions.[15] The ages estimated using heated teeth from persons aged 44 to 63 years were 115 years to 152 years, respectively, for total amino acid, considerably older than the actual ages. The ages estimated from the ratio for soluble peptide extracted with 1M HCl were 42 to 61 years, respectively, being approximately the same as the true ages. Thus, it is essential to use soluble peptide for the estimation of age using burned teeth.

ACKNOWLEDGEMENTS

We thank Dr T Yamamoto from the Biological Laboratory of our College for valuable suggestions.

Address for correspondence:
Dr Susumu Ohtani
Department of Forensic Dental Medicine
Kanagawa Dental College
82 Inaoka-cho, Yokosuka Kanagawa 238
Japan

REFERENCES

1. Schroeder RA, Bada JL. Glacial-postglacial temperature difference deduced from aspartic acid racemization in fossil bones. Science 1973; 182: 479-82.

2. Matsuura S, Ueta N. Fraction dependent variation of aspartic acid racemization age of fossil bone. Nature 1980; 286: 883-4.

3. Helfman PM, Bada JL. Aspartic acid racemisation in dentine as a measure of ageing. Nature 1976; 262:279-81.

4. Ogino T, Ogino H, Nagy B. Application of aspartic acid racemization to forensic odontology: post mortem designation of age at death. Forensic Sci Int 1985; 29: 259-67.

5. Ohtani S, Yamamoto K. Racemization velocity of aspartic acid in dentine. Jpn J Legal Med 1990; 44: 346-51.

6. Ohtani S, Yamamoto K. Age estimation using the racemization of amino acid in human dentin. J Forensic Sci 1991; 36: 792-800.

7. Ohtani S. Yamamoto K. Estimation of age from a tooth by means of racemization of an amino acid, especially aspartic acid-comparison of enamel and dentin. J Forensic Sci 1992; 37: 1061-7.

8. Ritz S, Schutz HW, Peper C. Postmortem estimation of age at death based on aspartic acid racemization in dentin: its applicability for root dentin. Int J Legal Med 1993; 105: 289-93.

9. Ohtani S. Age estimation by aspartic acid racemization in dentin of deciduous teeth. Forensic Sci Int 1994; 68: 77-82.

10. Mornstad H, Pfeiffer H, Teivens A. Estimation of dental age using HPLC-technique to determine the degree of aspartic acid racemization. J Forensic Sci 1994; 39: 1425-31.

11. Ohtani S. Estimation of age from dentin by using the racemization reaction of aspartic acid. Am J Forensic Med Pathol 1995; 16: 158-61.

12. Ohtani S. Estimation of age from the teeth of unidentified corpses using the amino acid racemization method with reference to actual cases. Am J Forensic Med Pathol 1995; 16: 238-42.

13. Ohtani S, Sugeno H, Marumo T and Yamamoto K. Two cases of age estimation from teeth of burned body using amino acid racemization. Jpn J Legal Med 1989; 43: 191-7.

14. Ohtani S. Estimation of age from dentin by utilizing the racemization of aspartic acid: influence of pH. Forensic Sci Int 1995; 75: 181-7.

15. Bada JL, Shou MY. Kinetics and mechanism of amino acid racemization in aqueous solution and bones. In Hare PE, Hoering TC and King K Jr (Ed.), "Biogeochemistry of amino acids", John Wiley & Sons: New York, 1980: 235-55.

16. Ohtani S, Yamamoto K. Estimating age through the amino acid racemization of acid-soluble dentinal peptides. Jpn J Legal Med 1990; 44: 342-5.

SEQUENCING MITOCHONDRIAL DNA FROM A TOOTH AND APPLICATION TO FORENSIC ODONTOLOGY

Y. Yamada[1,2], H. Ohira[1], H. Iwase[2], T. Takatori[2], M. Nagao[2], S. Ohtani[1]
1. Department of Forensic Dental Medicine, Kanagawa Dental College, Yokosuka, 238, Japan
2. Department of Forensic Medicine, Faculty of Medicine, University of Tokyo, 113, Japan

ABSTRACT

Genetic identification can be complicated by long intervals between the time of death and examination of tissues, and sometimes only bone and teeth may be available for analysis. Several investigators have described the isolation of nuclear DNA from these materials, but all have indicated that the DNA is significantly degraded. Recently, the polymerase chain reaction (PCR) and direct DNA sequencing have enabled rapid and reliable characterization of specific highly polymorphic DNA sequences from different individuals. Above all, mitochondrial DNA sequences offer several unique advantages for the identification of human remains. The isolation of mtDNA from a tooth and the symmetrical PCR amplification and direct DNA sequencing of its most polymorphic regions are reported. **(J Forensic Odontostomatol 1997; 15: 13-16)**

Keywords: odontology, identification, human remains, mitochondrial DNA, D-loop, sequencing

INTRODUCTION

The present study was designed to investigate the forensic value of teeth as a source of DNA for analysis. A long interval between the time of death and subsequent examination is associated with decay of soft tissues from which DNA could have been extracted, and this makes it extremely difficult to perform active identification of individuals by this method. After soft tissues have decayed, hard tissues, i.e. bone and teeth, remain as materials for use in possible identification.

Bone marrow and dental pulp cells are sources of DNA. A comparison of these tissues has shown that bones, particularly thick bones, contain abundant marrow cells and are therefore suitable for the extraction of large amounts of DNA. However, in severely damaged or decomposed bodies which must be identified, bones are often broken, allowing the invasion of bacteria and the destruction of the soft tissues. Although teeth contain far fewer cells than bones, the pulp cavity containing pulp cells provides a stable environment for DNA. Dental enamel protects the tooth and the pulp cavity against decay and the invasion of external bacteria and it has also been reported that the high content of hydroxyapatite in the dentine enhances the stability of DNA.[1]

At present, the main procedure used for DNA analysis in forensic medicine is the polymerase chain reaction (PCR), for which the purity rather than the amount of DNA is important. Therefore, in bodies requiring forensic evaluation, teeth which are less likely to be contaminated by foreign substances are superior to bones as a source of DNA.

We have previously reported[2] that the pulp degenerates and disappears rapidly in teeth kept in a wet environment, making it difficult to obtain intact DNA, and in fact experience shows that the dental pulp has already degraded or been eliminated in almost all cases. When DNA is available the PCR procedure is adopted because mitochondrial DNA (mtDNA) offers several unique advantages for the identification of remains. Firstly, mtDNA is present at high copy numbers relative to genomic DNA, generally more than 500 copies per cell and thus is more likely to be present in very small samples. Secondly, mtDNA is maternally inherited, an individual sharing the mtDNA sequence of his or her siblings, mother, and so on. Thirdly, mtDNA is haploid, simplifying direct sequencing of the amplified DNA product and finally, mtDNA contains highly polymorphic regions.[3] Yoshii *et al*[4] in a survey of 100 Japanese individuals, reported the presence of 66 types of mtDNA.

We have found that mtDNA from teeth is sufficiently intact to allow PCR amplification of the D-loop region.[5]

CASE REPORT

A burned, headless human body was found in a parking lot in Tokyo in January, 1996 (Fig.1) and four months later a skull was found buried in the ground near the parking lot (Figs.2, 3 and 4). We were asked by the police to give an expert opinion of whether the skull and the headless body were those of the same person, which we did using DNA analysis from tissue samples collected at autopsy.

Figure 1. A burned, headless human body (Yamada et al)

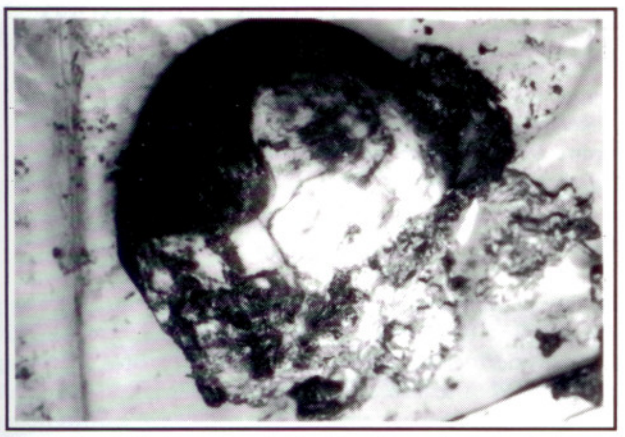

Figure 2. Skull of woman missing from home four months (Yamada et al)

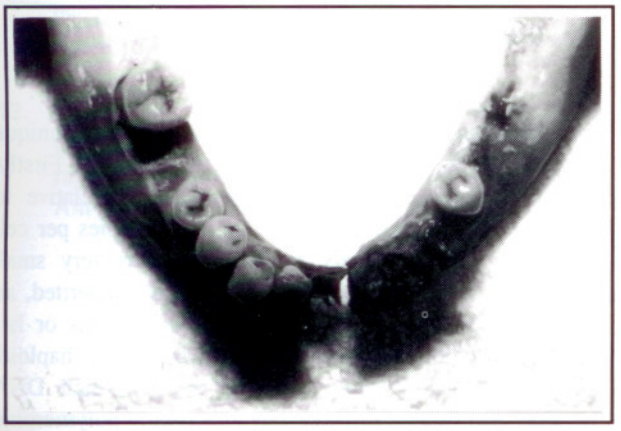

Figure 3. Occlusal view of the victim's teeth (Yamada et al)

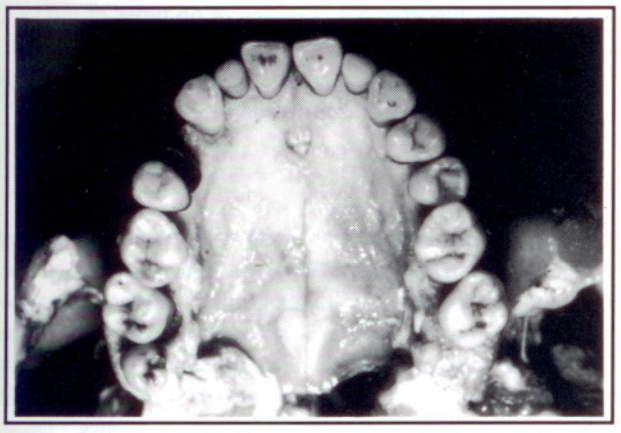

Figure 4. Pieces of mandible (Yamada et al)

MATERIALS AND METHODS

Muscle tissue was removed from the leg of the body, and the first premolar tooth from the skull. DNA extraction from the soft tissue specimen (500mg) was performed using the proteinase K, phenol/chloroform extraction method and ethanol precipitation while DNA was extracted from the tooth using the Chelex mini-column method.[7] DNA was extracted from the dentine by removing material from the dentinal surface, and the tooth was then cut (not crushed) to reveal the pulp cavity.[5] Pieces of the tooth were placed in 50ml of 0.5M EDTA solution and incubated at room temperature for 2 days. Following this the sectioned tooth was incubated in lysis buffer containing 10mM Tris (2-amino-2-hydroxymethyl-1,3-propanediol), 0.1M NaCl, 5mM EDTA, proteinase K (0.1mg/ml) and 2% SDS (sodium dodecyl sulfate) overnight at 50°C. After the tooth had been digested, the DNA was extracted using phenol-chloroform followed by ethanol precipitation and it was then dissolved in TE (10mM Tris, 0.1mM EDTA) to form a DNA solution.

Finally the DNA solution was added to a Chelex-100 mini-column (pH 8.0), centrifuged and the extract was purified. A Chelex-100 chelating ion* exchange resin was purchased and DNA quality and quantity were estimated in agarose testgels and DNA fragments were visualized by ethidium bromide.

(a) Amplification of mtDNA and chromosal hypervariable loci and ABO gene locus

The amplifications were carried out with the following primers and conditions which are summarised in Tables 1 and 2. Each amplification sample of the total volume of 50 microlitre contained a 10ng template, 10mM Tris-HCl, pH 8.3, 50mM KCl, 0.2mM each dNTP, 1.5mM $MgCl_2$, 1 unit Taq polymerase and 25pM each primer. Amplifications were carried out by using the Program temp control system PC-700.**

(b) Automated Solid Phase Sequencing

The DNA was sequenced with an ABI Prism Dye

* Bio-Rad Laboratory, Richmond, CA USA

** Astec, Fukuoka, Japan

Locus	Primer	Sequence
mt4524	452-1	CAT GGG GAA GCA TTG
	452-2	TTA GCT ACC CCC AAG TGT
HUMTH01[8]	TH01/1	GTGGGCTGAAAAGCTCCCGATTAT
	TH01/2	GTGATTCCCATTGGCCTGTTCCTC
D1S80[9]	MCT118/1	GAAACTGGCCTCCAAACACTGCCCGCCG
	MCT118/2	GTCTTGTTGGAGATGCACGTGCCCCTTGC
HLADQA[10]	GH26	GTGCTGCAGGTGTAAACTTGTACCAG
	GH27	CACGGATCCGGTAGCAGCGGTAGAGTTG
ABO[11]	0-1	CACCGTGGAAGGATGTCCTC
	0-2	AATGTCCACAGTCACTCGGC
	B-1	TGGAGATCCTGACTCCGCTG
	B-2	GTAGAAATCGCCCTCGTCCTT

Table 1. Primer sequences
Superscripts: references HUMTH01: human tyrosine hydrolxyase gene, intron 1 D1S80: pMCT118 VNTR

Locus	Denaturation		Annealing		Extension		No. cycles	Final extension at 72°C (min)
	°C	s	°C	s	°C	s		
mt452	94	60	55	60	72	120	30	7
HUMTH01	94	60	54	60	72	60	35	7
D1S80	94	60	65	60	72	60	29	10
HLADQa	94	60	58	60	72	60	30	7

Table 2. A summary of the PCR methods to obtain results at the mt452, HUMTH01, D1S80, HLADQσ, and ABO loci: PCR cycling conditions

```
sequence No.  16111     120       130       140       150
Body(muscle)  ............................................
Skull(tooth)  ............................................
control       CCACCATGAATATTGTACGGTACCATAAATACTTGACCACCTGT
      160       170       180       190       200
....................................................
....................................................
AGTACATAAAAACCCAATCCACATCAAAACCCCCTCCCCATGCTTACAAGCA
   210       220       230       240       250
................T...................................
................T...................................
AGTACAGCAATCAACCCTCAACTATCACACATCAACTGCAACTCCAAAGCCA
 260       270       280       290       300       310
...............................T....................
...............................T....................
CCCCTCACCCACTAGGATACCAACAAACCTACCCACCCTTAACAGTACATAG
        320       330       340       350       360
........A..........................................C
........A..........................................C
TACATAAAGCCATTTACCGTACATAGCACATTACAGTCAAATCCCTTCTCGTC
      370       380       390       16399
....................................
....................................
CCCATGGATGACCCCCCTCAGATAGGGGTCCCTTGA
Shown are replaced nucleotides detected from two samples.
Symbols: ( • ) no replacements.
```

Table 3. Showing mtDNA sequences (16, 111~ 16,398) within the body(muscle), the skull(tooth) and control.[5]
Shown are replaced nucleotides detected from two samples.
Symbols (•) no replacements

Terminator Cycle Sequencing Core Kit** and used according to the manufacturer's instructions. Electrophoresis and sequence analysis was performed with an ABI model 373A sequencer.☆

RESULTS AND DISCUSSION

Mitochondrial DNA sequences were amplified from tooth DNA. The identities of the skull and body were then determined by combining PCR amplification with direct DNA sequencing and a total of 288 base pairs in the D-loop region of the target mtDNA were amplified by PCR. Table 3 shows the mtDNA sequences from the body, the skull (tooth) and control.[10] Yoshii *et al.*[4] reported the presence of 66 types of mtDNA, but this case's type was very rare and is found in only one out of 100 individuals. The sequences were thus consistent with one another demonstrating that the body and skull were those of the same person while the identity of the body was established by comparing the mtDNA sequence with that of maternal relatives (data not shown). Furthermore, the amplification of chromosal hypervariable loci and ABO gene locus showed that both tissues were identical (Table 4).

Locus	Body (muscle)	Skull (tooth)
HUMTH01	6, 6*	6, 6*
D1S80	18, 30*	18, 30*
HLADQα	1.3, 1.3†	1.3, 1.3†
ABO	BO†	BO†

Table 4. The comparative typing of nuclear markers HUMTH01 and D1S80 which allele designation ws derived from the bands observed in the allelic ladder (HUMTH01 ; Promega, Madison, WI, D1S80; Perkin-Elmer, Norwalk, CT). HLADQa alleles detected by PCR-ASO probes (Perkin-Elmer). ABO genotype is determined by the intersection of possible genotypes predicted by KPN I(O allele) and Alu I (B allele) digestion.
** These are the allele types (number of repeat units)*
† These are the genotypes

Dental identification is traditionally based on a comparison of antemortem with postmortem dental charts and x-rays. In cases where this is impossible the usefulness of mtDNA sequencing is undisputed and it should become more widely known and used as an adjunct in forensic odontological practice.

Address for correspondence:
Dr Y Yamada
Department of Forensic Dental Medicine
Kanagawa Dental College
Yokosuka 238
Japan

** Astec, Fukuoka, Japan
☆ Perkin Elmer, Norwalk, CT USA

REFERENCES

1. Ginther C, Tarver LI, King MC. Identifying individuals by sequencing mitochondrial DNA from tooth. Nat Genet 1992; 2: 135-9.

2. Utsunomiya J. Sex determination by PCR method of teeth - special reference to influence sea-water and underground- (in Japanese) Kanagawa Shigaku 1994; 29: 217-26.

3. Yamada Y, Tsukamoto T, Zhang W-D, Oono T, Iwadate K, Nagao M, Takatori T. Experience with cloned mitochondrial DNA analysis to forensic practice. Jpn J Legal Med 1994; 48: 44-8.

4. Yoshii T, Takeda E, Akiyama K, Ishiyama I. Sequence polymorphism of mitochondrial DNA and its forensic application (in Japanese) Jpn J Legal Med 1995; 49: 242-50.

5. Yamada Y, Yamamoto K, Yoshii T, Ishiyama I. Analysis of DNA from tooth and application to forensic dental medicine. Jpn J Legal Med 1989; 43: 420-3.

6. Polymeropoulos MH, Xiao H, Rath DS, Merril CR. Tetranucleotide repeat polymorphism at the human tyrosine hydrolase gene (TH). Nucleic Acids Res. 1991; 19: 3753.

7. Kasai K, Nakamura Y, White R. Amplification of a variable number of tandem repeats (VNTR) locus (pMCT118) by the polymerase chain reaction (PCR) and its application to forensic science. J Forensic Sci. 1990; 35: 1196-200.

8. Gyllensten UB, Erlich HA. Generation of single stranded DNA by the polymerase chain reaction and its application to direct sequencing of the HLADQα locus. Proc. Natl. Acad. Sci. USA 1988; 85: 7652-6.

9. Lee JCI, Chang JG. ABO genotyping by polmerase chain reaction. J Forensic Sci. 1992; 37: 1269-75.

10. Anderson S, Bankier AT, Barrell BG, DeBruijn MHL, Colson AR, Drouin J, Eperon IC, Nierlich DP, Roe BA, Sanger F, Schreier PH, Smith AJH, Staden R, Young IG. Sequence and organization of the human mitochondrial genome. Nature. 1981; 290: 457-65.

A SIMPLE MEANS OF DEMONSTRATING SKULL FRACTURES USING RADIOGRAPHIC ALTERED IMAGE GEOMETRY

J.K. Wong[1], B. Blenkinsop[2], D. Chiasson[2], R.E. Wood[1]
1. Princess Margaret Hospital, Toronto, Ontario, Canada
2. Office of the Chief Coroner, Province of Ontario, Canada

ABSTRACT

Multiple comminuted fractures distributed around a skull and resulting from repeated weapon impacts were poorly demonstrated on conventional lateral and upper frontal radiographs. This was due to superimposition of contralateral fractures, normal anatomy and tangential projection angles. Simple modification of a standard dental x-ray generator and manipulation of projection geometry allowed isolation of individual fractures with improved image quality. The above technique lends itself to cases in which soft tissue remains to obscure the detail of multiple fractures during clinical observation. **(J Forensic Odontostomatol 1997; 15: 17-21)**

Keywords: skull fractures, radiography, forensics

INTRODUCTION

Radiography in forensic investigations is a basic and indispensable technique with applications ranging from fingerprint imaging to skeletal/dental identification of bodies reduced to states beyond recognition.[1] It remains the simplest and most efficient method of detecting fractures and may indicate their type, age, number and cause. It may furthermore provide a pre-examination record of a specimen which could obviate charges that fractures were caused in an autopsy procedure itself.[1]

Skull fractures may involve the cranium, skull base, or both and are further classified in Table 1.[2-5] Skull regions susceptible to fracture include the vertical and orbital plates of the frontal bone, middle cerebral fossa, centre of the squamous temporal bone and centre of the occipital fossa.[2] Diastatic fractures usually involve sagittal and coronal sutures.[2] Most skull injuries are associated with automobile accidents[4] with frequencies being highest in middle aged males, equal in infancy and old age and with overall increases coinciding with summer outdoor activity.[2]

Radiography is commonly utilized in assessing head trauma,[5,6] using axial, half axial, occipito-frontal and lateral films as standard views for assessment of the vault and skull base.[2,3,5-9] Tangential views for depressed fractures may also be used.[3-8]

In investigating criminal acts causing skull fracture radiography may allow determination of weapon shape, impact direction, nature of force involved (e.g. penetrating) and timing of fractures.[10] It is as decisive as a post-mortem examination in these respects and has proved useful both for living or deceased subjects.[10] Manipulation of projection geometry, also called "lateral magnification techniques" has been used in mammography to improve image size and sharpness of breast micro-calcifications.[11]

The purpose of this paper is to describe a technique in which an altered dental x-ray generator can be used to provide radiographs which are superior to conventional skull views.

MATERIALS AND METHODS

The specimen utilized was that of a partially skeletonized female homicide victim in which the skull had multiple fractures upon the frontal, temporal and both parietal regions (Figs.1 and 2).

I) Simple or closed: broken bone does not pass through skin
II) Compound or open: broken ends of bone pass through skin
1) Complete: involving both inner and outer cortices
2) Incomplete: involving only the inner or outer cortices
a) Linear: extending parallel to suture line of bone
i) Fissure: extending into cortex but not entire bone
Vertical
Horizontal
Oblique
ii) Fissure type (along a pre-existing suture)
iii) Cleft or splint (extending from the suture to internal aspect of the bone)
b) Commuted: crushed into a number of pieces, often the result of massive trauma to the vault[2-5]
i) Splintering type: with thin, sharp bone fragments
c) Depressed: fragments depressed below the surface of the skull
d) Buttonhole: bone perforated by a missile
e) Diastatic: separation of bones at a suture

Table 1. Classification of Skull Fractures

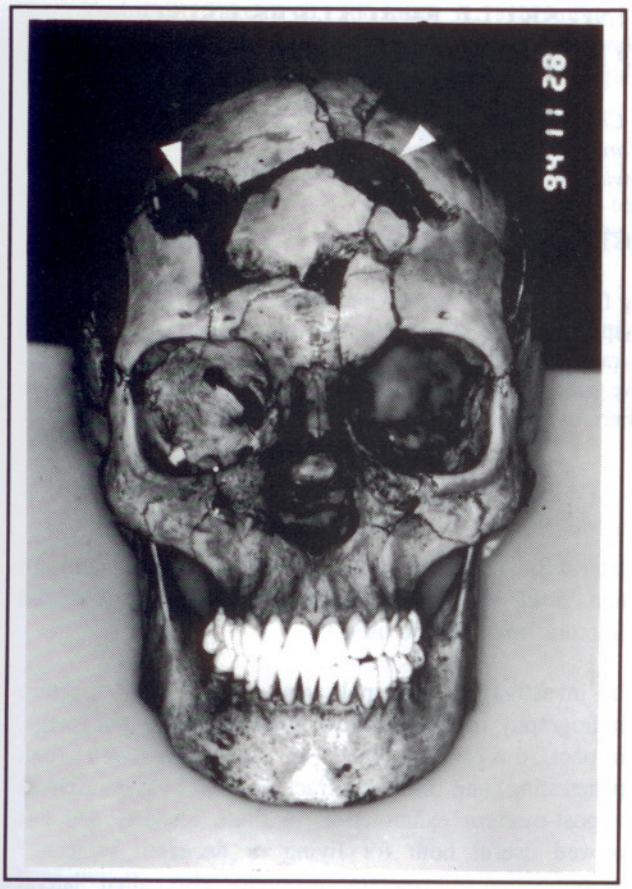

Figure 1. Frontal view of de-fleshed skull of homicide victim. Note the two fractures visible on the frontal surface (arrows) which were obscured prior to de-fleshing

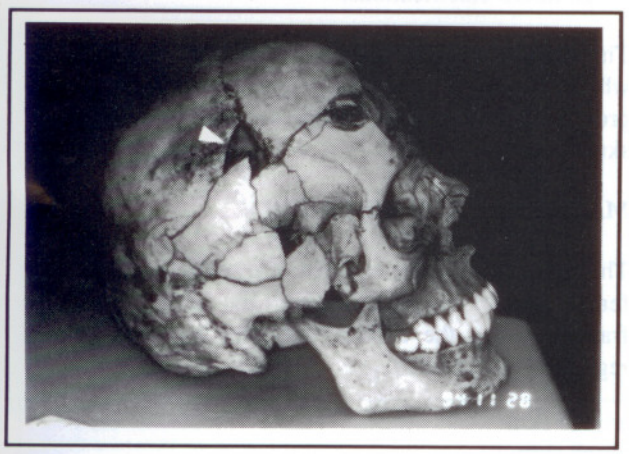

Figure 2. Right lateral view of same homicide victim. Note avulsed portion of temporal-parietal junction and numerous fracture lines (arrow).

A standard dental x-ray generator was modified by removing the collimator and Kodak Lanex Regular screens* were used in conjunction with 8 × 10 inch TMG 400 film. The specimen was positioned with the particular fracture zone in question against the film cassette, the x-ray port positioned perpendicular to the film and the central ray aligned with the area to be imaged. The anode was then moved toward the specimen to a distance of 6cm, where exposures of 1/6th second at 70kVp and 10mA were used.

* Kodak Corporation, Rochester, New York, USA

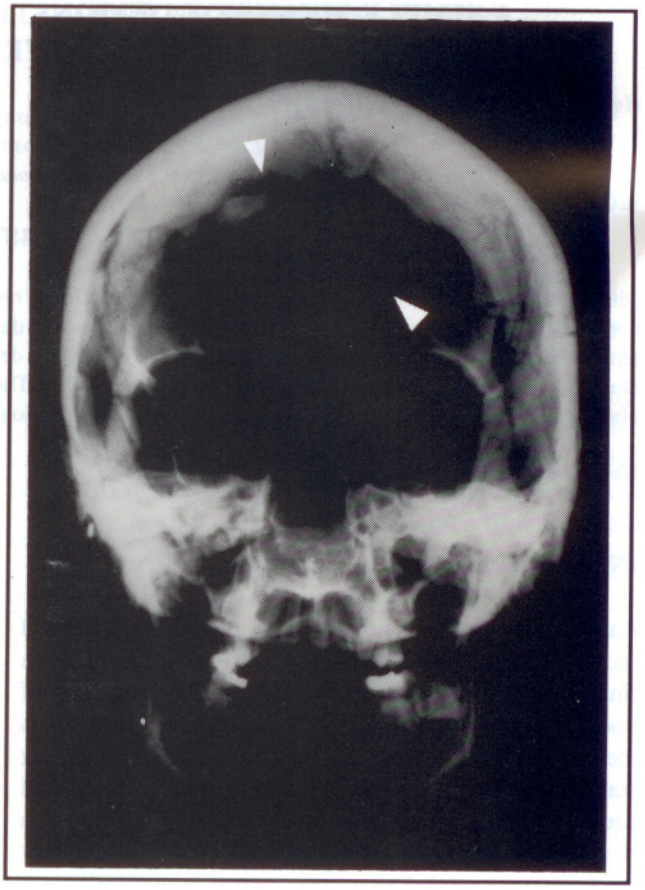

Figure 3. Antero-posterior projection of skull using standard medical x-ray equipment. Note multiple visible fracture lines in the supra-orbital region (arrows).

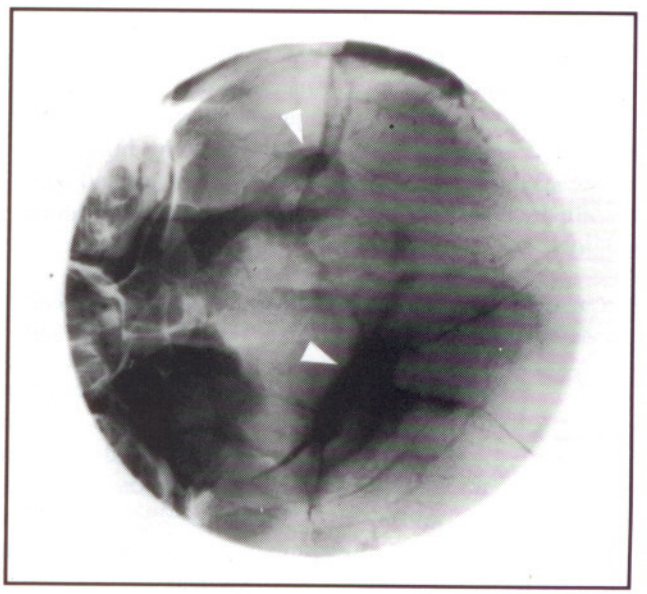

Figure 4. Modified dental x-ray generator projection of supra-orbital area depicted in Fig. 3. In this view the two separate skull fractures located in the supra-orbital region are well visualised and the occupital features and anatomical structures have been selectively removed by magnification (arrows).

Using the said apparatus a standard right lateral horizontal (0 degrees to the orbitomeatal plane, 90 degrees to film) and an upper frontal projection (-25 degrees to the orbitomeatal plane, 90 degrees to film) were exposed.

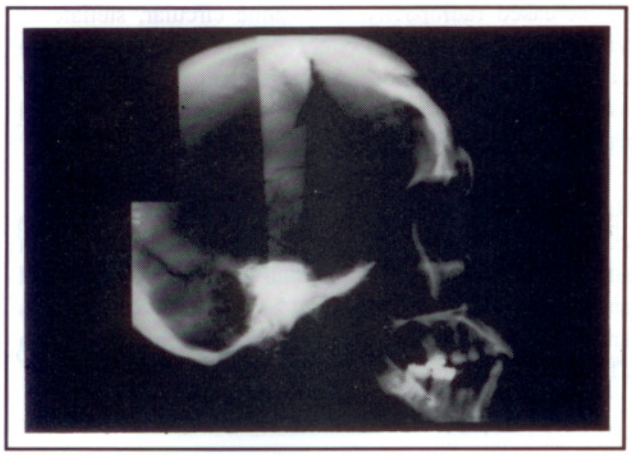

Figure 5. Right lateral projection of skull using standard medical equipment. Note numerous confusing vascular markings and superimposed skull fracture lines about the avulsed segment.

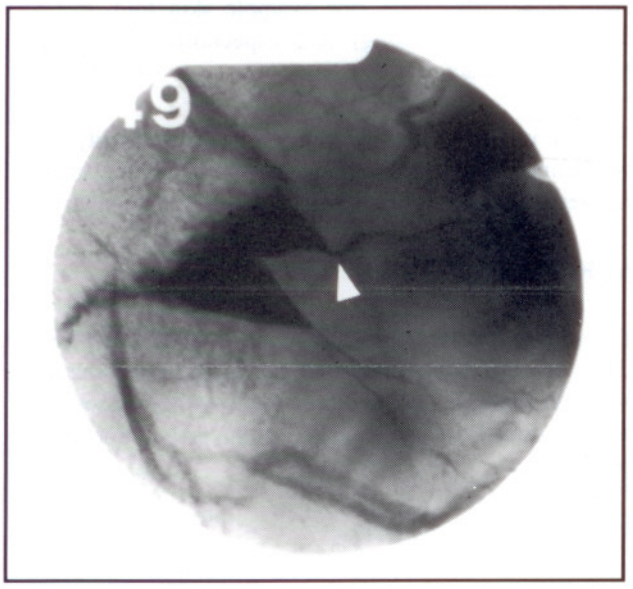

Figure 6. Modified dental x-ray generator projection of the right temporal region showing avulsed portion and associated fracture lines (arrow). These were not well visualised in the standard medical x-ray projections.

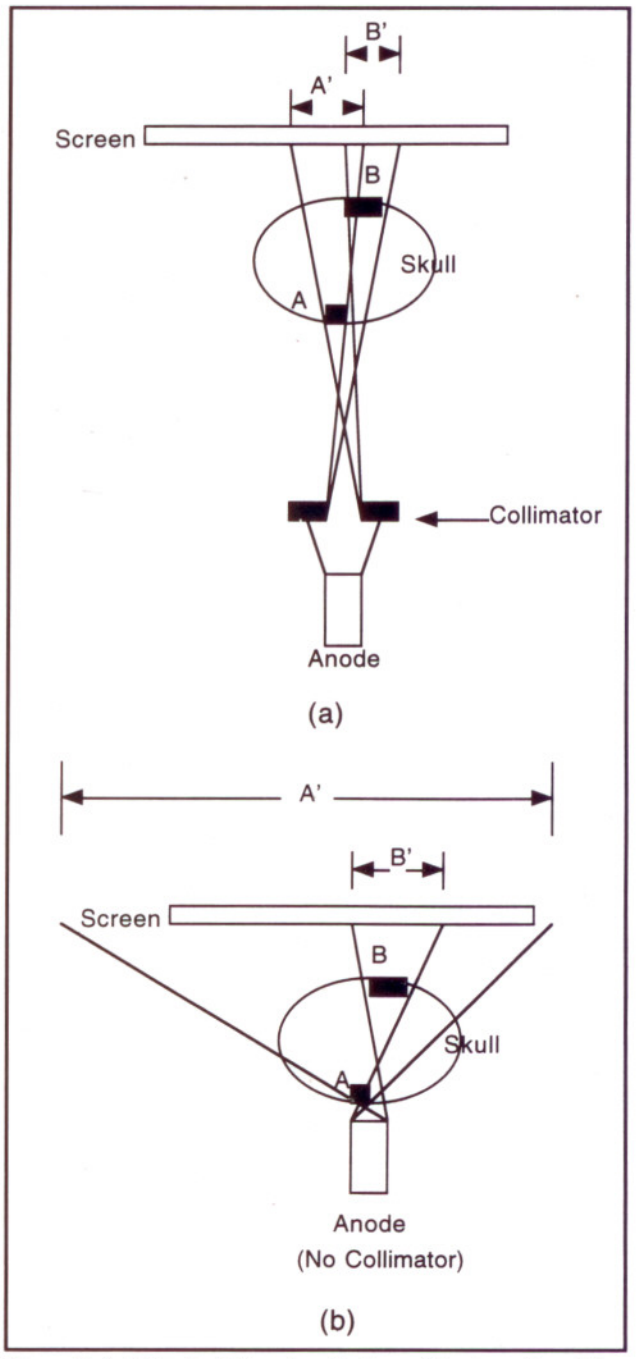

Figure 7. (a) Standard lateral projection of fractures A and B results in superimposition of images A' and B'. (b) Keeping fracture B closest to film, x-ray head with collimator removed is moved toward skull effectively reducing the focal spot-object A distance. The result is an increase in size and penumbra of image A' and improved quality and isolation of image B'.

RESULTS

Images obtained through standard lateral and upper frontal views are depicted in Figs.3 and 5. Radiographic images obtained through use of the modified dental generator to isolate specific fractures are depicted in Figs.4 and 6.

Improved resolution of fine linear fissure fractures from lines of force of weapon impact is apparent (Fig.4). Shadows from a depressed occipital fracture are not present in Fig.4 while a projection of the left orbital frontal bone barely visible in Fig.3 is apparent in Fig.4. The overall pattern of both frontal fractures is demonstrated more clearly in Fig.4 than in Fig.3 and superimpositions from distant parts of the skull are removed in this technique.

The fractures surrounding the avulsed segment of temporal bone seen in Fig.1 and depicted radiographically in Fig.6 are free from superimposed transverse and vertical linear fractures seen in the views produced by the medical x-ray generator. It is also far more simple to deduce which radiolucent lines are fractures and which are vascular markings (Figs. 5 and 6).

DISCUSSION

The ability to obtain an acceptable radiograph of fractures depends very much on the particular positioning of focal

spot, object and film. In the case of a skull, placement of the fractured side closest to the film gives the best delineation[3,4] while on the opposite side, they appear less well defined and of poor contour.[4] They appear grossly magnified on the tube side and the degree of penumbra increases, resulting in a large ill-defined image.[11]

In cases involving multiple comminuted fractures on opposing sides, the resultant image with a standard frontal or lateral view becomes a complex intermixing of shadows with any one particular pattern difficult to isolate.

Undesirable fracture and vascular images are eliminated in this technique by simple manipulation of imaging geometry (Fig.7). Decreasing the focal spot to object distance increases magnification and penumbra of proximal structures rendering the resultant image free of distracting shadows. Film side structures remain sharply defined as object to film distance is comparatively small.

In criminal investigations, imaging precise shapes and sizes of gross and fine fractures allows delineation of weapon dimensions and lines of force application.[10] The value of the above technique is apparent in situations in which skin, hair and flesh remain and obscure direct observation of fractures with the naked eye. Standard views may initially indicate general positions of fractures but individual patterns are better resolved with this technique.

Compound comminuted fractures often accompany trauma and penetrating wounds (e.g. sharp objects or bullets). Depressed fractures like those seen here (Fig.6) result from localized direct force and may appear as areas of increased radiopacity, or assume circular, stellate or triangular shapes.[2,4,5] Rounded defects such as those visible in the supra-orbital region (Fig.4) are usually associated with weapons such as a hammer or a fall against a sharp object.[4]

1. Arterial and Venous Grooves:
Middle meningeal, middle temporal artey and deep temporal arteries.
Vessels are generally not as sharply cut. Meningeal grooves spread out in a fan shaped fashion from foramen spinosum. Anterior branch of middle meningeal more distinct and runs parallel to coronal suture. Vascular markings diminish in size from base of skull to cranial vertex.
2. Fissures:
Lateral fissure of foramen magnum, lateral interparietal fissure median occipital fissures, parietal fissures.
3. Sutures:
Occipito- and parieto-mastoid, squamosal, mendosal, frontal.
Have a sclerotic saw-tooth or irregular appearance.
4. Diploetic Spaces:
Resemble branches of a tree directed upward from cranial base.
They lack splintering and are much wider and more irregular than fracture lines.
5. Mastoid Cells
6. Focal Thinning of the Inner Table

Table 2. Normal anatomy often confused with fractures

Radiographically, fractures may appear short and straight, curved or irregular.[4,5] A fracture may follow the line of a suture[4] and therefore it is essential to be aware of normal anatomical structures that could be mistaken for fractures and characteristics that may help to differentiate the two (Table 2).[2-6]

CONCLUSIONS

Image quality of individual skull fracture patterns is compromised in standard skull views by superimposition of normal anatomy, foreign bodies, soft tissue lacerations, extra cranial structures (for example skin fold, matted hair, ear lobe, dressing) and especially contralateral comminuted fractures.[5,6]

Fracture isolation and improvement in image quality may be obtained by simple modification of a standard dental x-ray generator and manipulation of projection geometry.

ACKNOWLEDGEMENTS

The authors wish to express their appreciation to Mr Keith Oxley and Ms Meta Antolin of the graphics arts section, Mr John Jackson and Ms Carol Tullis of library services and Ms Anna Dias for assistance in the preparation of the manuscript.

Address for correspondence:
Dr RE Wood
Princess Margaret Hospital
610 University Avenue
Toronto, Canada, M5G 2M9

REFERENCES

1. Graham D. The use of x-ray techniques in forensic investigations. Longman Group Limited, 1973: 142.

2. Stewart WH. Skull fractures roentgenologically considered. New York: Paul B. Hoeber Inc., 1925: 64.

3. Young BR. The skull, sinus and mastoids: A handbook of roentgen diagnosis. Chicago: The Year Book Publishers Inc., 1948: 327.

4. Birzle H, Bergleiter R, Kuner EH. Radiology of trauma. Stuttgart: George Thieme Publishers, 1978: 372.

5. Greenbaum EI. Radiology of the emergency

6. Berquist TH. Diagnostic imaging of the acutely injured patient. Baltimore: Urban & Shwarzenberg, 1985: 250.

7. Kimber P. Radiography of the head. Edinburgh: Churchill Livingstone, 1983: 200.

8. Korach G, Vignaud J. Manual of radiographic techniques of the skull. Paris: Masson Publishing, 1981: 144.

9. Du Boulay GM. Principles of x-ray diagnosis of the skull (Second ed.). London: Butterworth & Co Ltd, 1980: 400.

10. Simpson K. The use of radiography in the investigation of crime. Radiography 1980; 46: 14-6.

11. Shiabel H, Ventura A, Frere AF. A formal study of lateral magnification and its influence on mammographic imaging sharpness. Medical Physics 1994; 21: 271-6.