Effects of long-term steroid therapy on the results of dental age estimation using pulp/tooth ratio

Akiko Kumagai [1], Taiko Oishi [2], Hanako Sato [2], Yukiko Sugiyama [2], Toshiro Sato [2], Roberto Cameriere [3]

[1]Division of Forensic Odontology and Disaster Oral Medicine, Department of Forensic Science, Iwate Medical University, Iwate, Japan. [2]Division of Preventive Dentistry, Department of Oral Medicine, School of Dentistry, Iwate Medical University, Iwate, Japan. [3]Department of Medicine and Health Science "Vincenzo Tiberio", University of Molise, AgeEstimation Project, Campobasso, Italy

Corresponding author:
kumagaia@iwate-med.ac.jp

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KEYWORDS

Dental age estimation, Pulp chamber constriction, Long-term steroid therapy, Steroidal osteoporosis, Forensic odontology.

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ABSTRACT

Forensic age estimation is performed by assessing pulp chamber constrictions due to physiological age-related changes on dental radiographs; however, the estimated ages occasionally deviate from the actual ages. In particular, long-term steroid users tended to demonstrate pulp chamber constrictions in all teeth. As this is uncommon among younger age groups, caution should be exercised when evaluating pulp chamber constriction. This study investigated the estimated ages of eight steroid users by applying the ratio of the pulp area to the total tooth area from canine radiographs. Patients in their 30–40s were examined at a dental outpatient clinic for the prevention or treatment of adverse events associated with the use of bisphosphonates to prevent steroidal osteoporosis and radiographs were obtained. The pulp and tooth areas were measured, and the estimated age was determined using regression formulas calculated from the canine teeth of the general Japanese study participants. The mean absolute error between the estimated and chronological ages of the patients was 19.24 years for the upper canines and 17.69 years for the lower. The root mean square error was 23.18 years for the upper canines and 20.00 years for the lower. The estimated ages were far from the actual ages of the steroid users. When estimating the age of an unidentified individual who has a pulp chamber constriction that is inconsistent with other forensic physical findings, this information may assist in predicting their medical background.

INTRODUCTION

Accurately estimating the age of unidentified bodies assists forensic investigations in identifying corpses, which is an important aspect of forensics. Human teeth exhibit prominent physiological age-related changes and are often examined for age estimation using forensic medical techniques. Since Gustafson proposed a method for age estimation in 1950,[1] many forensic scientists have conducted studies to accurately estimate an individual's age at death. However, in real-world forensic cases, various factors affect the accuracy of such estimates. Although an individual's age can be estimated using various methods, it is crucial to select one that has been scientifically proven to yield accurate results.

Cameriere et al. assessed pulp chamber constriction caused by the age-related physiological addition of secondary dentin and

estimating age using dental radiographs of the Italian population by means of a quantitative method.[2] This method has been applied by many researchers. Different methods for assessing pulp chamber constriction have been reported in various countries.[3–7] However, the estimated ages occasionally deviate from the actual ages, especially in cases of prominent pulp chamber constriction throughout the jaw due to long-term steroid (LTS) use. As widespread pulp chamber constriction due to LTS use is inconsistent with the natural changes in the pulp area caused by aging, forensic scientists must exercise caution when estimating age using this aspect. Therefore, we verified the estimated age by applying the pulp/tooth ratio (PTR) of eight Japanese patients who had received LTS therapy to two regression formulae: the Japanese model that was derived from general Japanese subjects gathered in this study, and Cameriere's method.[2]

MATERIALS AND METHODS

Derivation of regression formulae for the Japanese population

We retrospectively examined 440 digital periapical radiographs of healthy Japanese canine teeth obtained for examination and treatment at the dental center of Iwate Medical University. The subjects consisted of 172 men and 268 women with an age range of 26–84 years (upper canines: men 68, women 122; lower canines: men 104, women 146) for the derivation of the regression formulae for the Japanese population (Table 1).

Table 1. Number of subjects distributed according to age group.

Part of teeth	Men (age)	Women (age)	Total (age)
Upper	68 (29–84 y)	122 (26–80 y)	190 (26–84 y)
Lower	104 (29–84 y)	146 (31–80 y)	250 (29–84 y)
Total	172 (29–84 y)	268 (26–80 y)	440 (26–84 y)

In the cases of patients in their fifties or older, it is difficult to determine whether the cause of the pulp cavity constriction is due to LTS use or age-related changes. Therefore, relatively young patients, 30–40 years old, were selected for this study. The digital dental X-ray equipment used was ALULA-TM (Asahiroentgen Ind. Co., Ltd, Kyoto, Japan) and MAX-DC70 (J. MORITA MFG. Corp., Kyoto, Japan). Each image was converted to a JPEG and saved, following the method described by Cameriere et al.[2] The lasso tool in ImageJ (open source imaging processing program, National Institute of Mental Health, Bethesda, MD, USA) was used to measure the pulp areas and overall teeth in labial and buccal views of the upper and lower canines. All measurements were performed by the same observer. To test the intraobserver reproducibility, a random sample of 30 periapical radiographs was reexamined after an interval of two months. The intraobserver reproducibility of the measurements was assessed using the intraclass correlation coefficient (ICC).

Verification of the estimated age based on radiographs of the canines of Japanese patients who underwent long-term steroid therapy

Eight patients, all aged 30–40 years, had been administered a steroid, 1–75 mg converted to prednisolone (PSL), for more than four years. The LTS users were not included as general participants. All patients were examined at a dental outpatient clinic for the prevention or treatment of adverse events associated with the use of bisphosphonates to prevent steroidal osteoporosis (Table 2), and digital radiographs were obtained. All canines selected from the patients in this study were healthy and did not have any treatment marks. After measuring the canine pulp and tooth areas and calculating the PTR, we applied the PTR to the regression formulae (Japanese method) based on the Japanese population and Cameriere's method based on Italian subjects,[2] and compared the differences between their chronological and estimated ages in terms of the mean absolute error, root mean square error, and interquartile range.

The protocols for this study were approved by the Ethics Committee of the Iwate Medical University (approval no. 01352).

Table 2. List of Japanese patients on long-term steroid (LTS) therapy

Case No.	Age	Sex	Diagnosis	*Term / Quantity of PSL dosage
1	41	man	interstitial pneumonia with dermatomyositis	4 years / 15–75 mg
2	46	man	pancolitic ulcerative colitis	11 years / 5–50 mg
3	39	man	primary myelofibrosis	4 years / 30–50 mg
4	45	woman	scleroderma	6 years / 12.5 mg
5	46	woman	systemic lupus erythematosus	13 years / 5–50 mg
6	35	woman	systemic lupus erythematosus	15 years / 5–70 mg
7	47	woman	systemic myasthenia gravis	5 years / 5–20 mg
8	39	woman	systemic myasthenia gravis	18 years / 1–60 mg

PSL: Prednisolone.
* Medical agents administered as steroids were converted to PSL.

RESULTS

ICC analysis showed reliable results for intraobserver differences between the paired sets of measurements performed on the re-examined periapical radiographs [ICC (1,1) = 0.775].

The regression model for the upper and lower canines, based on the measurements of the Japanese population, yielded the following regression formula, which explained 83.8% of the total variance (R^2 = 0.702).

Age = -344.921*PTR upper-36.727*PTR lower+86.608

When only the upper canines were considered, the regression formula, which explained 92.0% of the total variance (R^2 = 0.846), was:

Age = 97.169-510.365*PTR upper

When only the lower canines were considered, the regression formula, which explained 89.3% of the total variance (R^2 = 0.798), was:

Age = 88.535-429.939*PTR lower

The accuracies are listed in Table 3. When Cameriere's method was applied to the Japanese subjects, the mean absolute errors between the estimated and chronological ages for the upper and lower canines, upper only, and lower only, were 5.87 years, 4.02 years, and 3.83 years, respectively; the root mean square errors were 7.19 years, 4.69 years, and 4.78 years, respectively; and the mean interquartile ranges were 7.75 years, 7.48 years, and 6.48 years, respectively. When the Japanese regression formulae were applied to the Japanese subjects, the mean absolute errors between the estimated and chronological ages for the upper and lower canines, upper only, and lower only, were 4.32 years, 3.84 years, and 3.68 years, respectively; the root mean square errors were 5.46 years, 4.53 years, and 4.59 years, respectively; and the mean interquartile ranges were 5.33 years, 7.16 years, and 5.92 years, respectively. The regression formulae based on the Japanese population yielded more accurate estimates; however, it was proven that the regression formulae used in Cameriere's method also estimated the ages of the Japanese participants with reasonable accuracy.

The results of the verification of the accuracy of the estimated ages from the canines and chronological ages in LTS users were as follows: when using the regression formulae by Cameriere et al., the mean absolute error between the estimated and chronological ages of the eight patients was 20.45 years for the upper and lower canines, 20.52 years for the upper only, and 16.63 years for the lower only. Moreover, the root mean square error was 24.25 years for the upper and lower canines, 24.50 years for the upper only, and 19.06 for the lower only. When using the regression formulae from the Japanese subjects, the mean absolute error between the estimated and chronological ages of steroid users was 17.81 years for the upper and lower canines, 19.26 years for the upper only, and 17.69 years for the lower only. The root mean square error was 20.55 years for

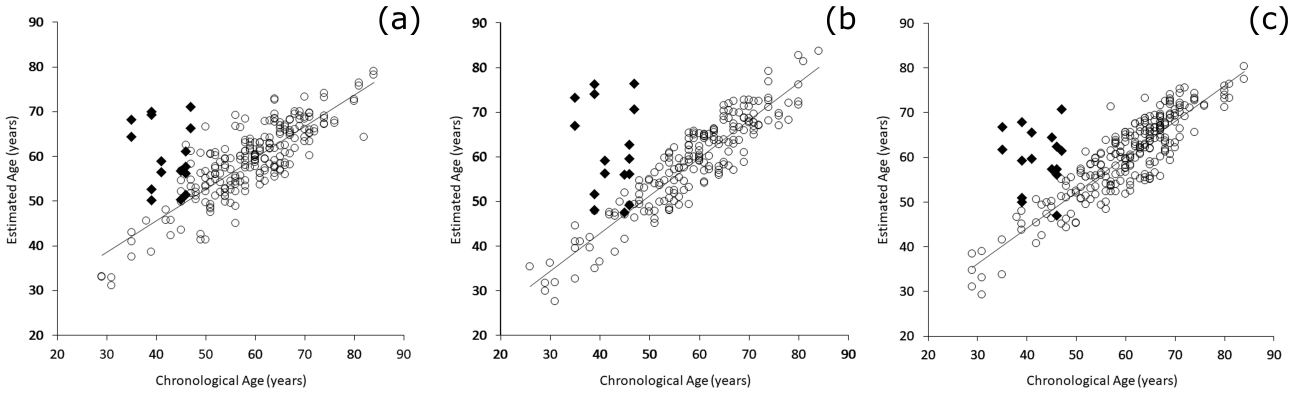
the upper and lower canines, 23.18 years for the upper only, and 20.00 years for the lower only. The estimated ages of LTS users were far from the regression line derived from the general study subjects as well as their chronological ages (Figure 1).

Table 3. List of Japanese patients on long-term steroid (LTS) therapy

Part of teeth	Subjects	MAE		RMSE		MIQR	
		CM	JM	CM	JM	CM	JM
Upper and Lower	General	5.87	4.32	7.19	5.46	7.75	5.33
	PSL user	20.45	17.81	24.25	20.55	9.63	5.74
Upper	General	4.02	3.84	4.69	4.53	7.48	7.16
	PSL user	20.52	19.26	24.50	23.18	8.55	8.18
Lower	General	3.83	3.68	4.78	4.59	6.48	5.92
	PSL user	16.63	17.69	19.06	20.00	4.14	3.85

MAE: mean absolute error
RMSE: root mean square error
MIQR: mean interquartile range
CM: Cameriere's method
JM: Japanese method in the present study
LTS user: long-term steroid user

Figure 1. Plots of data and regression line using a simple linear regression model of Japanese canines. Open circle: General subjects, Filled rhombus: LTS user. (a) upper and lower canines, (b) upper canines, and (c) lower canines.



DISCUSSION

Synthetic glucocorticoids, namely, steroids, are used for their strong anti-inflammatory and immune-boosting effects. Their main action is to suppress the production of prostaglandins by suppressing the expression of enzymes involved in arachidonic acid metabolism and to suppress the production of various inflammatory cytokines. Steroids are used to treat many diseases, including collagen diseases, respiratory diseases, allergies, kidney diseases, blood diseases, and post-transplant rejection, and it is commonly used to treat autoimmune diseases.[8,9] However, other effects may lead a variety of adverse reactions, such as neuropsychiatric symptoms, anemia, muscle weakness, and increased susceptibility to infection, diabetes, dyslipidemia, hypertension, and atherosclerosis.[10] In particular, steroid-induced osteoporosis, which is caused by a decrease in bone strength, is more prone to fractures than primary osteoporosis even if bone density is maintained, and significantly reduces the quality of life of patients.[11] The mechanism of

onset is said to be inhibition of differentiation into osteoblasts[12], suppression of osteoclast apoptosis[13], decreased calcium absorption and increased excretion[14,15], and the suppression of various hormone secretions by excess steroids, which promotes bone resorption.[16]

Bisphosphonates (BP) are administered as one of the medicines for treating steroid-induced osteoporosis, and many patients undergoing steroid therapy visit dental clinics for oral examinations and oral care to prevent the onset of osteonecrosis of the jaw, a side effect of BP. The causes of osteonecrosis of the jaw by BP include the fact that it is covered only by a thin oral mucosa, a large number of resident bacteria present in the oral cavity, and inflammation due to dental infections or invasive dental treatment that easily spread to the jawbone.[17] However, within the scope of the authors' search, there have been no reports of BP having had an effect on the teeth.

All the patients in this report were undergoing LTS therapy to alleviate the symptoms of their primary disease, and dental X-rays were taken for oral management to prevent osteonecrosis of the jaw caused by BP, which confirmed pulp cavity constriction. However, the effect of steroids on teeth has not been clarified. The reported effects of LTS use on teeth include hyperesthesia-like symptoms[18,19] and pulp chamber constriction.[20] The mechanism of the onset of pulp chamber constriction has not yet been fully determined, but it is thought to involve a phenomenon in which steroids enhance the expression of the dentin sialophosphoprotein gene and type 1 collagen, as well as alkaline phosphatase activity.[21,22] Moreover, steroids are fat-soluble and therefore distributed in a wide range of tissues; however, the proteins they bind to and their binding strength varies depending on the type of steroid.[8,9]

Pulp chamber constriction due to steroids depends on when the steroid is used, how long it has been used, and the dosage.[20] Most patients in this study started using steroids when they were 20–30 years old. The estimated age of subjects with a history of high-dose steroid injections was extremely high. Among the subjects of this study, especially those in their twenties who had repeatedly increased and decreased their steroid dose for 18 years, we found pulp area stenosis that was inappropriate for their age (Figure 2). Furthermore, when we compared the previous and present results of patients who had radiographs taken twice, several years apart, we observed that the pulp area had clearly narrowed in a short period (Figure 3). In the upper canines, the estimated age did not match the actual age when the age estimation method derived in this study was applied.

Participants who showed a narrow pulp area included those with histories of mental illnesses such as depression, neurodevelopmental disorders, schizophrenia, and bruxism. There were also unknown causes besides LTS use; therefore, various aspects must be duly considered when performing forensic age estimation.

Figure 2. X-ray image findings of the patient (case No. 8), 39 years old, woman. Left: Upper left incisors and canine. Right: Lower left incisors and canine. The pulp area on each tooth is narrow, and the findings are not congruent for the age of the patient.

Figure 3. X-ray image findings of the lower left lateral incisor and canine in the same patient (case No. 5). Left: Two years ago, 44 years old. Right: Present findings, 46 years old. The pulp area of each tooth's crown section in particular has narrowed rapidly in just a few years.

CONCLUSION

The estimated ages of the Japanese LTS users were significantly different from their actual ages and from the regression line derived from the general study participants. For cases that require age estimation in the forensic field, the identity of the person is unknown and there is no way to determine their medical history. In estimating age, if the findings of the relevant person's pulp area on the radiograph are inconsistent with other physical forensic findings, the information may assist in predicting their medical background.

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