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Age Estimation of Iron Age Skeletons from Qareh Tape of Sagzabad Iran On the Basis of Pulp/Tooth Ratio Method

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Abstract:

Estimating the age of death has always been a challenging subject in bioarchaeology . Various methods of age estimating based on bone and dental indicators have been devised. Pulp/tooth area ratio has been employed as a method of age estimation. With aging pulp volume reduces due to the deposition of secondary dentine. Although this method has been tested and validated by researchers, it has not been investigated in ancient human remains . The purpose of this study was to evaluate the accuracy of age estimation method on human remains by assessing pulp/tooth area ratio, especially in cases where other age estimation indicators (pelvis and skull) are not useful. In this study, 21 canines from 9 buried individuals in the eastern cemetery of Iron Age II and III of Qareh Tape of Sagzabad , were selected for age estimation; The age of human skeletons were estimated using four different methods such as closure of skull sutures in two different ways, dental wear and eruption of third molars. Based on the obtained data and comparing them with each other, the method of calculating pulp/tooth area ratio should be used cautiously during the period of third molar eruption.

Keyword: *Bioarchaeology, Age Estimation, Pulp/Tooth Ratio, Qareh Tape of Sagzabad, Iranian Central Plateau.*

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1. Introduction

Faced with a human skeleton, the first questions asked are whether the individual was male or female and how old he or she was at the time of the death. Estimation of age at death is an essential part of reconstructing information from skeletal material and one of the main tasks of forensic and archaeological research, but the aims and human material of these disciplines are different. Person identification is an important aspect of Forensic medicine and dentistry. Age is an important factor in clinical practice; research and courts of law (De Luca et al., 2010). In the forensic context, age determination is not only a prerequisite for stating that an individual has officially died; it also is the basis for investigating crimes, mass disasters or war crimes. Judicial requirements, time and the accuracy of the age range are the basics in developing identification procedures (Hoppa and Vaupel, 2002). Estimation of age is equally important for forensic and archaeological purposes, but archaeological investigations can also reveal the age and sex of skeletons, including the demographic composition of including the demographic composition of these groups who used a particular cemetery. When an individual’s sex and age are combined with contextual information such as artifacts, grave details and burial location, they can be the center of research on mortuary practices of past societies (Milner et al., 2008).The biggest criticism of paleo-demography which is defined as "*a rigorous study of the demographic structure of past populations represented by skeletons*"(Milner et al., 2008) focuses on how to estimate the age of dead individuals by skeletal remains, especially in adults (Milner et al., 2008).Another criticism is: the meaning of “age estimation” were considered too imprecise for a demographic signal to penetrate stochastic noise (Bocquet-Appel and Masset, 1982). Central to paleo-demography it is the generation and interpretation of skeletal age distributions, as this information offers key insights into the demographic composition of a particular population and possibly differential mortality based on age. Reconstruction of mortality patterns is essential for an understanding of the living condition which human populations faced in historic ecosystems (Hoppa and Vaupel, 2002).

In the paleo-demographic studies, estimating the age of adults is a much bigger problem than doing the same for adolescents. Adolescents' ages can usually be estimated with acceptable error, using tooth crown and root development, tooth eruption and epiphyseal closure (Milner et al., 2008). Adult age can be reported either as point estimates or interval spanning several years or more. The open-ended terminal intervals, which are commonly used (e.g., 50+ years) obviously cover much of the possible human lifespan, although in preindustrial societies, old-age attrition was certainly higher than in modern times (Milner et al., 2008). Today, no osteologist believes that the exact age of an adult skeleton that looks like they might be about a certain age could be determined with equal confidence to the same age interval. Each skeleton has its own degree of error depending on the suite of traits that happen to be present (Milner et al., 2008).

The most widespread techniques of adult age determination, according to body preservation, are based on the analysis of macroscopic characteristics of various skeletal structures (Cunha et al., 2009): the pubic symphysis (Suchey and Katz, 1998; Todd, 1921), the auricular surface of the ilium (Lovejoy et al., 1985), the sternal rib ends (Iscan and Loth, 1989) or the endo- and ectocranial sutures (Galera et al., 1998; Meindl and Lovejoy, 1985). However, these specific skeletal structures are commonly subjected to the taphonomic processes and are often not recovered or are too damaged to contribute to a satisfactory osteological analysis.

Thanks to recent and relevant advances of physical anthropology, traditional methods have been improved and new techniques were recently proposed (De Luca et al., 2010). Dental and bone material from each exhumed body or parts of the body are helpful in the age estimation process. There is more or less interdependence among time and many natural changes of the human body which can be used for determination of age at death. Patterns of aging are detected on both macroscopic (direct observation and radiological examination) and microscopic levels (Iscan and Kennedy, 1989; Brothwell, 1981). The ageing of human remains is based upon a detailed knowledge of biological changes that occur during development, growth and maturation (Spalding et al., 2005). The exact chronology of these changes is dependent upon physiological variations in any individual (Whittaker and MacDonald, 1989). Skulls and teeth can provide a lot of information about age at death and that is the reason why there are many age determination methods based on cranial and dental features

(Vodanovic et al., 2011). In addition, in secondary burials, especially when the skull has been re-burned without infracranial skeleton, Ageing methods using the pelvis are useless. On the other hand, in mass burials, attributing the correction of different bones to an individual, is too difficult. Therefore, in these cases, the estimated age for each skull can be considered as the estimated age for each individual (regardless of the attribution of infracranial skeleton to each individual). Skeletal remains from archaeological contexts suffer from several biasing factors such as post-mortem changes, taphonomy and various burial practices depending on age, sex and social status of the deceased person while currently anthropological methods of age determination, however reveal several possibilities of inaccuracy (De Luca et al., 2010).

Of all the body parts used in age estimation, teeth are the least affected by any taphonomic process (De Luca et al., 2010). Dental structures are becoming more and more useful, because of their resistance to physical and chemical agents. They are still preserved even when most of the bones have been destroyed, mutilated or affected by other taphonomic agents (Lucy and Pollard, 1995). With the move towards improving existing techniques of age at death estimation, more accurate age-related changes in teeth have recently been re-examined as an alternative to skeletally based techniques. Although there are many dental methods based on macroscopic, microscopic and biochemical analysis of teeth, some of which are very complicated and expensive and imply partial destruction of the tooth (Gustafson, 1950; Martín de las Heras, 1999; Ohtani, 1991; Renz and Radlanski, 2006; D'Ortenzio et al., 2017) so, they are not normally used in bioarchaeology.

One of the best-known features of ageing is the reduction in size of the pulp chamber, caused by the continual secretion of dentinal Matrix by odontoblasts (physiological secondary dentinogenesis) (Solheim, 1992). Taking this into account, study of the apposition of secondary dentine by examining peri-apical X-rays of canines is beginning to supply very interesting results (De Luca et al., 2010).

Dentine is a living tissue containing odontoblasts which form the tooth and which, during a person's lifetime, for both physiological and pathological reasons, deposit layers of secondary dentine which gradually obliterates the pulp chamber (Bodecker, 1925; Vasiliadis et al.,1983).

Secondary dentin formation begins after the root is completed. Continuation of the secondary dentin formation appears to be a biological response to aging that can be used as a marker in estimating age (Hye-Mi Jeon et al., 2014). The mean rate of increasing dentinal thickness has been found to be 6.5 mm per year for the crown and 10 mm per year for the root. The effect of continuous dentine deposition is the progressive increase in dentinal thickness by 0.45 mm (17.1%) and 0.60 mm (24.3%) in the crown and root areas, respectively. The pattern of secondary dentine deposition varies with tooth type. As a regard to sex, no statistically significant differences are observed (Murray et al., 2002).

Secondary dentin deposition in teeth has been studied by several methods, one of which is the use of peri-apical X-rays of canines. Cameriere and his colleagues studied the relationship between the age at death and the ratio of the pulp/tooth area in peri-apical X-rays of upper (maxillary) and lower (mandibular) canines of several individuals in an identified Italian osteological collection (Cameriere et al., 2007).

Reliability is the degree to which a method produces the same results when it is used at different times, either by multiple observers or by the same observer. It can be tested by conducting inter-observer or intra-observer variation studies to determine error rates. Low inter - observer variation (or error) indicates high reliability(Adams & Byrd, 2002).

The main aim of this paper was to test the reliability of Cameriere's method in nine Iron age samples of unknown age at death, from Qareh Tape of Sagzabad and to examine its use in order to estimate the age of skeletal remains from archaeological contexts. This method, which was tested in a small sample of 9 identified mummies (14th century) from the Basilica of S. Domenico Maggiore (Cameriere et al., 2006) and a large Medieval sample from various archaeological sites (De Luca et al., 2010) is now tested for the first time on 3000-year-old skulls.

Another aim of the paper was to compare obtained results by various ageing methods using the skull. Many ageing techniques have demonstrated a general trend of over-ageing younger individuals and under-ageing older ones (Aykroyd et al., 1997). So, regarding the differential preservation of samples is a major source of bias in Paleodemographic parameters (Bell et al.,1996; Haglund and Sorg, 2002), these data can determine which of these methods tend to estimate high and low ages among all the procedures.

It is a fact that Paleodemographic samples tend to be small and are produced by a selective, and typically incompletely understood. The typically small size of samples is largely a result of investigating small communities—even if long-lasting and completely excavated, their cemeteries rarely held large numbers of graves. If we hope to learn something about the life experiences of people in the distant past, we must come to grips with the problems posed by small samples, including determining how large is large enough for a sample to tell us something meaningful about the demographic structure of past populations (Hoppa and Saunders, 1998). The alternative, to give up on most of human existence, does not seem acceptable (Milner et al., 2008).

2- Materials and Methods

In this study, we used the upper and lower canine teeth of the human remains, which recovered in the eastern cemetery of Qareh Tape of Sagzabad in 2016, 2017 and 2018. Qareh Tape of Sagzabad (N: 3964025-4157 E: 405304-85) is located about 7 kilometers north of the small town of Sagzabad, in the Qazvin province.

The Qazvin Plain, extends along the Alborz Mountains in this area for c. 60km. Composed of a series of alluvial fans, it provides a suitable place for farming and animal husbandry. The southern part of the plain is provided with water by the Hajee Arab alluvial fan, with the village of Sagzabad at its top. Excavations of settlement and residential structures at Qareh Tape were conducted in 2014–2015. During the survey aimed at defining the limits of Qareh Tape, which was conducted in May 2016, a Cemetery dated to the Iron Age II and III was found in the eastern part of the site. Excavations at the newly discovered cemetery were initiated in 2017, in order to establish an accurate chronology of the graves and to increase knowledge about the burial practices and cultural patterns in the region. A new 10×20m trench (No. 12) was opened 180 meters northeast from the main site. Here the archaeologists discovered a multi-layer cemetery with the uppermost burial context found at a depth of 128cm (context 12016) and the deepest over two meters below (355cm; context12048). Most of the documented graves were so-called chest or mudbrick four-sidewall graves. The majority of graves were multiple interment collective burials (contexts 12021, 12030, 12033, 12038, 12040 and 12047), with only one grave (context 12035) containing a single skeleton. Human remains were usually either partially or completely disarticulated, although occasionally preserved anatomical order was noted (context 12038).

In the majority of graves, the dead were buried with small ruminants, such as ovicaprids. Furthermore, in all burials various offerings were documented, such as gray, buff and red wares, bronze arrowheads and various metal ornaments (iron, bronze, shell, agate, limestone, glass paste). In one grave (context 12033) many cylinder seals were documented, which may indicate a high social status of the residents of Qareh Tape. According to the cultural material, the relative chronology of layers 1 to 5 (excavated in 2017 and 2018) covers the Iron Age II and III (1100–550 BC). Two radiocarbon dates corroborated this dating: context 12047A dated to 1050–895 cal. BC (Poz-114528) and context 12035 dated to 924–812 cal. BC (Poz-114529) (Trębicka, et al., 2019).

The condition of the bone remains is good; especially skulls and jaws, which, despite the antiquity of the finds, have been very well preserved. A subsample of 21 upper and lower canines in 9 individuals was analyzed (Tab.2).

The age of each individual (skull) is represented by four common methods including vault sites and lat. ant. sites suture closure (Meindl and Lovejoy, 1985), dental wear (Lovejoy, 1985), and the time of eruption of the third molars (Mincer et al., 1993). All of these methods are non-invasive methods and this is very important in the examination of ancient bones and teeth. Suture closure and tooth wear were chosen because they are simple methods with a long tradition of usage for age estimation.

Age estimation by occlusal tooth wear was done according to Lovejoy`s method (Lovejoy, 1985). All teeth present except the first molars (because of severe and non-physiologic tooth wear) were used for this study. Dental wears which is the attrition of the occlusal or incisal surface of teeth, caused by mastication, (Fig.1) is certainly the easiest and fastest, but also the least accurate of the dental methods (Brothwell, 1989; Kim et al., 2000, Fiorenza et al., 2018). It has been employed as an estimator of age for prehistoric populations since the beginning of the 20th century.

The third molar existence was examined on each skull in order to determine the phase of eruption. If the third molar erupted completely and its occlusal surface was on occlusal plan, it was considered that the person at the time of death was older than 21 years. If it was erupting (Fig. 2), it was considered that the person was between 15 and 21 years at the time of death. In overview, the eruptive stage of the third molar can be the only quantitative biologic variable that is available for estimating the age of a person in his late teens or early 20s whether a person is at least 18 years of age, then the empirical probabilities can prove useful, particularly if the molar is just starting or ending its crown-root development (Mincer et al., 1993).

Although a high proportion of cranial sutures fusion (Fig.3) are generally seen by increasing age, there is considerable variability in closure rates. Such variation reduces the value of suture closure patterns for age estimation (Masset, 1989). Information on suture closure is, however, useful when other criteria is unavailable or when used in conjunction with other attributes (Acsadi and Nemeskeri, 1970; Meindl and Lovejoy, 1985). by year of excavation, individual's code and canine teeth. Because of less geometric errors, digital periapical radiographs were taken as parallel technique (Fig.4). By calculating the ratio between the area of the dental pulp and the area of the tooth using Rhino software, the age of samples was estimated according to Cameriere's proposed formula as follow (Fig.5). For maxillary canine: $Age=100.598-544.433\times RA$ For mandibular canine: $Age=91.362-480.901\times RA$.

Estimation of age based on pulp/tooth area ratio (Cameriere method)

Each canine was prepared by a parallel radiographic technique and the Rhino software was used to calculate the area of the tooth and its pulp. The area of dental pulp, the tooth and the ratio of the pulp/tooth area (RA) each of the canine are given in Table 3. Then, according to the Cameriere formula (Cameriere et al., 2004; 2006; 2007) for the upper and lower canine the estimated age of each individual was obtained based on the upper or lower canine or both (Tables 4-5).

3. Discussion

Each age estimation methods introduced above is compared here with the pulp/tooth area ratio method in a diagram are shown in figures 6 to 9. In figure 6, the estimated age for each individual has been shown as a vertical line representing the minimum, maximum and average age, based on the Vault Sites suture closure method. The age estimation based on the pulp/tooth area ratio method is also shown as points separately for the maxillary and mandibular canine. As shown in the diagram, the estimated ages by the pulp/tooth area ratio method for individuals 12030.A, 12030.C, 12035.A, 12038.A, 12018.E are in the estimated age range by the Vault Sites suture closure method, but in individual 12018.F with the estimated age range 27- 42 years by the Vault Sites suture closure method, the pulp/tooth area ratio method underestimates the higher and lower bound for age at birth. On the other hand, in individual 12047.B, the Vault Sites suture closure method offers an age range of 30 to 48 years, the pulp/tooth area ratio method offers a much younger age and estimated to be around 15 to 22 years. Except for these two cases, the estimated age of the other data is consistent in both methods. In figure 7, the estimated age for each individual has been plotted as a vertical line representing the minimum and maximum and average age, based on the Lateral-Anterior Sites suture closure method. Estimation of age based on the pulp/tooth area ratio method has also been dotted in terms of the maxillary and mandibular canines. As shown in the diagram, in individuals 12030.A, 12035.A and 12018.E the estimated age by the pulp/tooth area ratio method are in the estimated age range based on the Lateral-Anterior Sites suture closure method. the; But in the three individuals 12047.B, 12018.F and 12030.C the estimated age by the present study, is less than the estimated range of the Lateral-Anterior Sites suture closure method and shows a few years difference.

The Estimated age for each individual based on dental wear is shown in a vertical line showing a time interval based on the Lovejoy’s standard (figure 8). It should be noted that dental wearing strongly depends on the diet, the food production and consumption techniques in the community. Since the wear of the first molars in this collection seems to be unusual and non-physiological and thus reflects older biological age, these teeth were abandoned in all cases. However, in most of these samples’ different teeth (anterior, canine, pre-molars and molars) show different ages, and therefore, unlike the standard of Lovejoy’s method, which is estimated at a 5-year interval, here total estimated intervals have been used, so in some cases have led to estimated approximately 25-year intervals. this can be seen in Figure 8, the estimated age according to the method under investigation in all of these samples except individual

12035.A is in the estimated age range by dental wear method. However, estimation of age by the pulp/tooth area ratio method in individual 12047.B comprises the lower limit of estimated range by dental wear method and in individual 12018.E consists of the upper limit of estimated range.

The estimated age of the individuals based on the third molar eruption with an average age of 18 years old is shown in figure 9. It should be noted that in cases of complete eruption of the third molar and its placement on the occlusal plan, the age over 18 years is shown as a straight line representing more than 18 years. The estimated age has been drawn as a vertical line over a period of 15 to 21 years old in cases of third molar incomplete eruption and non-positioning on the occlusal plan. Estimated ages based on the pulp/tooth area ratio method have also been shown as points, separating for the maxillary and mandibular canine. As shown in Figure 4, in individuals of 12030.A, 12035.A, 12038.A, 12047.B, 12018.E the estimated ages according to the research method is in the period of eruption or non-eruption of the third molar. In individual 12030.C, despite the lack of complete eruption of the third molar and estimation of age between 15 and 21 years, the estimated age by the research method shows three different ages; 22, 26, 30 years old. Although age 22 corresponds to a high limit of eruption of the third molar, but age 26 and 30 do not. Also, in the individual 12018.F the estimated age of the mandibular canine corresponds to the estimated age of the third molar, but the estimated age by the maxillary canine is 27, that is about six years older than the maximum of the third molar eruption interval.

4. Conclusions

According to the results of this study and comparing different methods of estimating age in different time intervals, it seems that in 15-21 years old, the age of third molar eruption, this tooth eruption is the most accurate method for estimating age. Other methods, in this interval, including skull suture closure, dental wear, and the pulp/tooth area ratio, are less reliable and better not to use. The methods of suture closure seams are also more accurate in the fourth decade of life (30 to 40 years old), than in other time intervals, while it is not accurate for estimating age at ages below 30 years and above 40 years old.

Dental wears, although severely affected by diet and eating habits, can be cited in many cases; although it should always be used with caution and with regard to all aspects (such as severe first-molar attrition in this population) and also should consider all of the available teeth. However, some samples offer a wide range of ages.

Finally, the method used in the present study (the pulp/tooth area ratio in canine method), proved to be potentially applicable but with some cautions as our analysis indicated it seems that with increasing age the applicability of this method tends to be more precise. However, further studies will need large sample size and more analysis if so, the estimation models provided would enable us to predict not only the age of ancient but the age of living peoples for the current forensic science.

5. Role of funding

grant from funding agencies in the public, commercial, or not-for-profit sectors.

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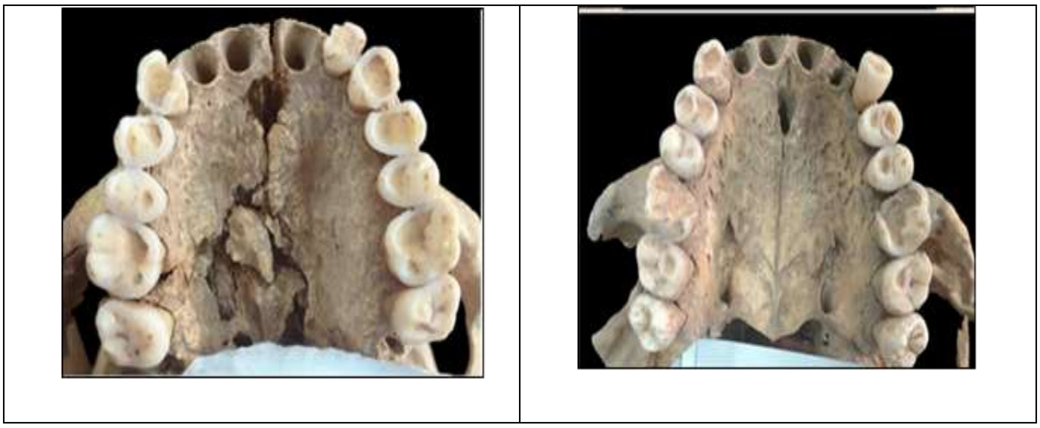

Figure 1- Dental wears method for age estimation

Figure 2-Third molar eruptions for age estimation

Figure 3- Cranial sutures for age estimation

Table 1. Estimated age of individuals by 4 research methods as mentioned in the text

Ind. code	3th molar eruption	Dental wear (Lovejoy method) *	Closure suture lat. ant. Sites	Closure suture Vault Sites
12030.A	21<	40-45	33-54	30-48
12030.C	15-21	16-30	31-52	27-42
12035.A	21<	35-40	31-52	30-48
12038.A	21<	20-45	31-52	30-48
12047.B	15-21	20-35	30-42	30-48
12018.E	21<	18-40	36-54	۳۳-۵۸
12018.F	15-21	18-45	31-52	27-42
12018.X	21<	40-45	-	-
95.A	21<	30-35	-	-
*Due to severe and non-physiologic tooth wear in first molars, these teeth are not considered in estimating age based on dental wear.				

Table 2. Specifications of the canine teeth samples

	Sample. Cod	Year of excavation	LL	LR	UL	UR	Sum
1	12030.A	2018			*	*	2
2	12030.C	2018	*	*		*	3
3	12035.A	2018	*	*	*	*	4
4	12038.A	2018			*		1
5	12047.B	2018		*	*	*	3
6	12018.E	2017		*	*	*	3
7	12018.F	2017		*		*	2
8	12018.X	2017	*	*			2
9	95.A	2016	*				1
Sum			4	6	5	6	21

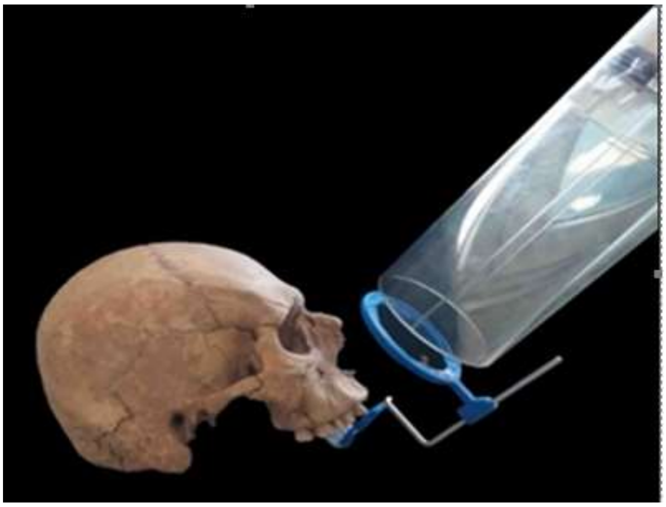

Figure 4- Digital periapical radiography, parallel technique

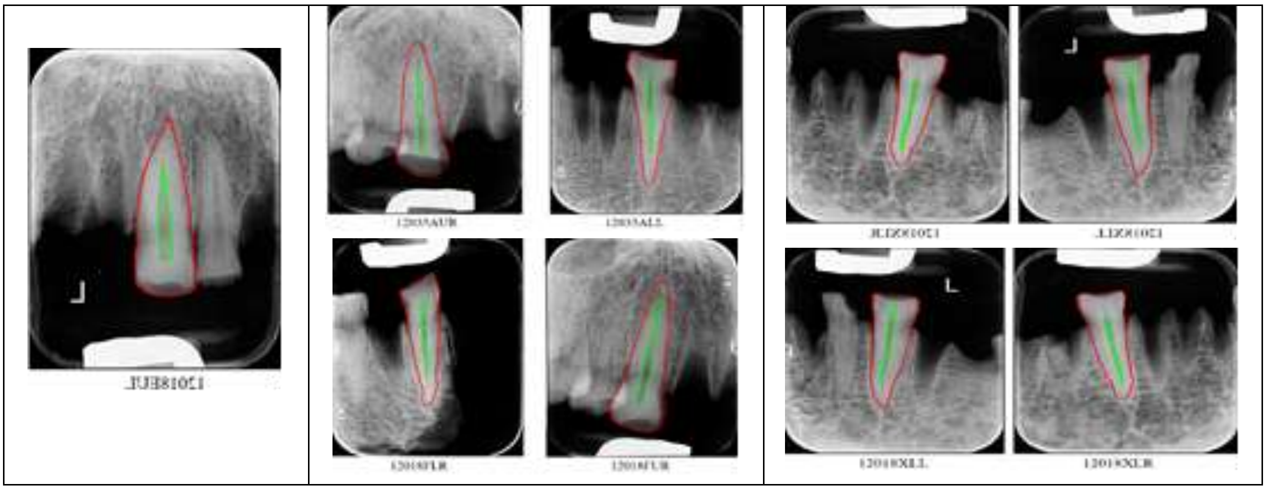

Figure 5. Calculating the area of the dental pulp and the area of the tooth in the Rhino software

Table 3 . Pulp and tooth area and pulp/tooth area ratio (RA), computed for individual samples

	Tooth code	Pulp area	Tooth area	RA
۱	12030.A. UR	0.066	0.603	0.109
۲	12030.A. UL	0.057	0.544	0.104
۳	12030.C. UR	0.077	0.538	0.143
۴	12030.C.L.R	0.056	0.429	0.130
۵	12030.C. LL	0.057	0.459	0.124
۶	12035.A. UR	0.050	0.481	0.103
۷	12035.A. UL	0.047	0.496	0.094
۸	12035.A.LR	0.036	0.381	0.094
۹	12035.A. LL	0.035	0.397	0.088
۱۰	12038.A. UL	0.080	0.611	0.130
۱۱	12047.B. UR	0.065	0.426	0.152
۱۲	12047.B. UL	0.072	0.496	0.145
۱۳	12047.B.LR	0.064	0.435	0.147
۱۴	12018.E. UR	0.052	0.460	0.113
۱۵	12018.E. UL	0.047	0.426	0.110
۱۶	12018.E.LR	0.034	0.335	0.101
۱۷	12018.F. UR	0.074	0.529	0.139
۱۸	12018.F.LR	0.056	0.367	0.152
۱۹	12018.X.LR	0.032	0.328	0.097
۲۰	12018.X. LL	0.038	0.345	0.110
۲۱	95A.LL	0.033	0.364	0.90

Table 4. Estimated age by maxillary canine (Age=100.598-544.433×RA)

	Tooth code	RA	Estimated age
١	12030.A. UR	0.109	41.254
٢	12030.A. UL	0.104	43.976
٣	12030.C. UR	0.143	22.743
۴	12035.A. UR	0.103	44.521
۵	12035.A. UL	0.094	49.421
۶	12038.A. UL	0.130	29.821
٧	12047.B. UR	0.152	17.844
٨	12047.B. UL	0.145	21.655
٩	12018.E. UR	0.113	39.077
١٠	12018.E. UL	0.110	40.710
١١	12018.F. UR	0.139	24.921

Table 5. Estimated age by mandibular canine (Age =91.362-480.901×RA)

	Estimated age	RA	Tooth code
١	28.844	0.130	12030.C.LR
٢	31.730	0.124	12030.C. LL
٣	46.157	0.094	12035.A.LR
۴	49.042	0.088	12035.A. LL
۵	20.669	0.147	12047.B.LR
۶	42.790	0.101	12018.E.LR
٧	18.265	0.152	12018.F.LR
٨	44.714	0.097	12018.X.LR
٩	38.462	0.110	12018.X. LL
١٠	43.271	0.100	95.A. LL

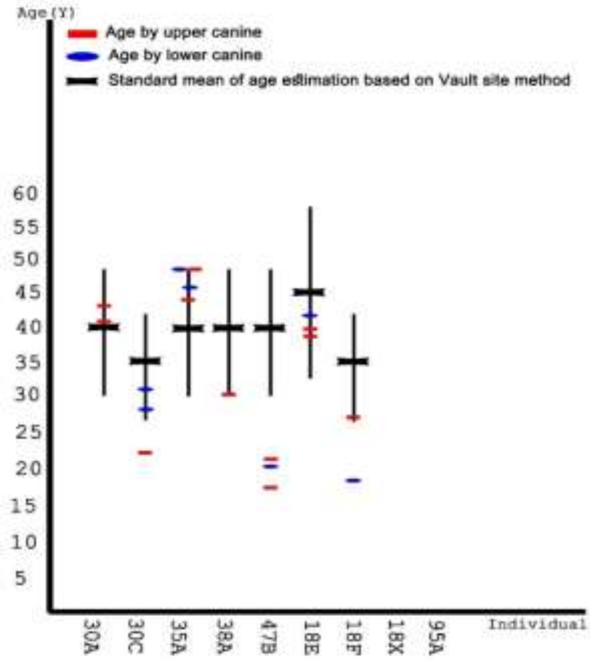

Figure 6- Comparison of estimated age: vault site method vs pulp/tooth ratio method

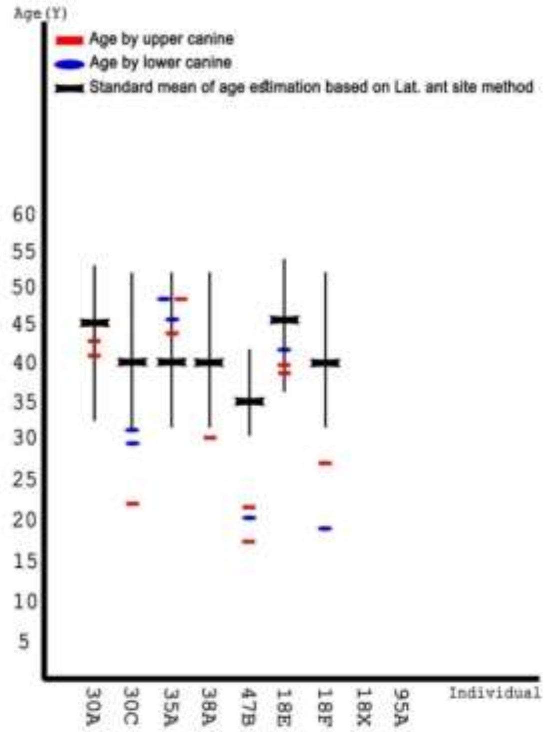

Figure 7- Comparison of estimated age: lat.ant. site method vs pulp/tooth ratio method

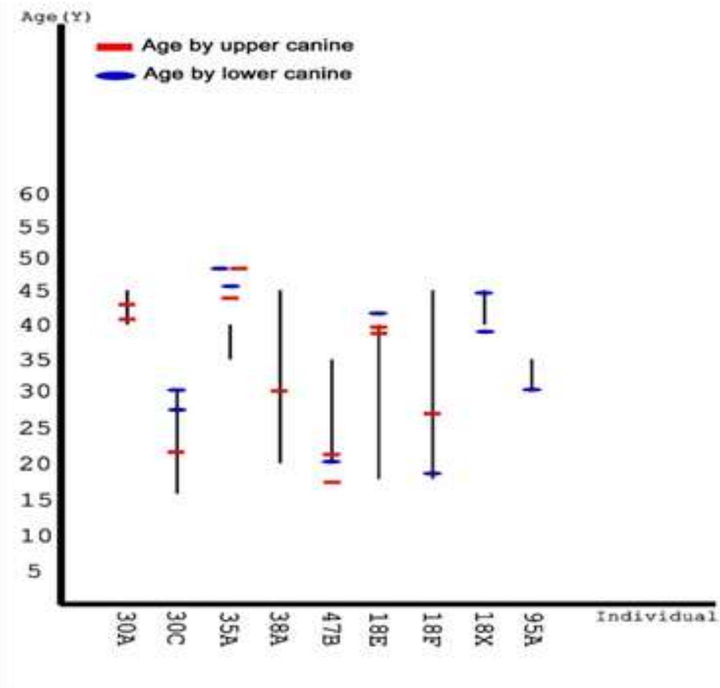

Figure 8- Comparison of estimated age: dental wear method vs pulp/tooth ratio method

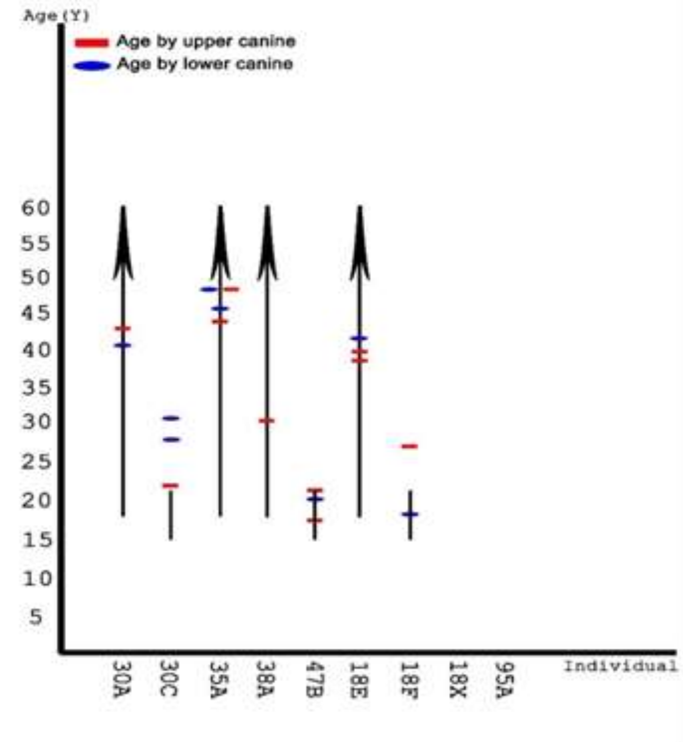

Figure 9- Comparison of estimated age: third molar eruption method vs pulp/tooth ratio

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