Dental Age Estimation of Ajnala Skeletal Remains: A Forensic Odontological Study*

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Abstract

The bones and teeth encrypt huge biological, chemical and molecular information about past life events of an individual. Teeth often survive almost all sorts of destructions and degradations and thus, help reconstruct individual life histories for comparatively longer periods of time than other human osseous remains. Dental attrition is the gradual and patterned loss of dental tissues during natural mastication which generally increases with the advancing age of an individual. In present study, three hundred eighty-nine (N=389) intact mandibular molars (176 first and 213 second) collected from Ajnala skeletal assemblage were considered for age estimations on the basis of their attrition levels using the average attrition stage (ASA) method proposed by Li and Ji (1-2). Thousands of unknown human skeletal remains were excavated non-scientifically from an abandoned well found underneath a religious structure at Ajnala, a north Indian suburb. The written accounts mentioned that 282 Indian-origin soldiers of the colonial army were killed in August 1857 and their corpses were dumped into the said abandoned well due to socio-political situations and sanitary reasons leading to their immediate burial (3-4). The mean age of the remains was estimated be 34.5 and 33.6 years from Ajmal et al (2) and Li and Ji (1) regression models, respectively. Preliminary anthropological, radiological, chemical and molecular results have also revealed that the recovered human remains belonged to adult males. Though attrition patterns are population-specific, the regression equations generated from dental attrition stages of Indian subjects are expected to provide more representative and valid age estimates of Ajnala skeletal remains. Present study findings may not be sufficient enough for age estimates of Ajnala remains for forensic purposes; these estimates may help in corroborative reconstruction of bioarchaeological information about these skeletal remains and may support age estimated from other analytical methods.

Keywords: forensic odontology; dental attrition; average stage of attrition; age estimation

** Author is responsible for language correctness and content.*

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Introduction

Teeth are the hardest structures of the human body which are resistant to some sorts of physical, chemical and biological degradations, taphonomic destructions, and traumatic insults (5). They usually serve as crucial indicators of biological identity (age, sex, ancestry, pathology, health status etc.) of an individual and can provide significant leads in forensic death investigations or bioarchaeological reconstructions. Teeth have remained the preferred and indelible sources of evidence about man's antiquity and have significantly contributed in revealing an individual's interactions with the physical, social, cultural and economic environment (6). Numerous studies have reported that anthropological, odontological or molecular characteristics of human dentition play pivotal role in establishing the identity of unknown human skeleton remains; the molecular methods provide more reliable results towards positive identity of such remains.

Attrition, a type of dental wear, is an age-dependent gradually advancing process which has been widely used for bioarchaeological reconstructions of the dietary habits, nutritional status and environmental conditions of past populations (7-8). Different stages of dental attrition can be assessed by examining the occlusal as well as incisal surfaces of a tooth. At the time of tooth eruption, molars and premolar crown has no attrition, and well-defined cusps are present on their occlusal surface with distinctly visible gullies. With the advancing age, the attrition gradually becomes more obviously visible, and the cusps become dull, faceted, and depressed in older ages. Finally, cusps ultimately get worn out to expose the dentine and the pulp chamber of the posterior dentition completely. Thus, dental attrition is one of several morphological changes that occur in the dental tissues with the advancing age or due to certain pathological or taphonomic alterations in anatomical features of the tooth.

All animal tissues, including bones and teeth, undergo age-progressive, irresistible and inevitable changes in their structural conformities. Attrition is a gradual and patterned loss of dental tissues like enamel and dentin during natural mastication and it is generally associated with the advancing age of an individual (7-11). Dental erosion, bruxism, medications, and environmental insults are considered as the contributing factors to the physiological process of tooth attrition. The age estimation of bioarchaeological or forensic samples becomes more important due to certain ethical, legal, moral or political reasons. Though, age-at-death estimation is one of the most complex, challenging and controversial components of the biological identity of a person to be established from unknown human skeletal remains; its accurate estimations can provide crucial leads in forensic death investigations. Anthropologically, attrition varies with the types of food eaten and geographic affiliations of an individual; the comparison of teeth characteristics among different population groups can help to understand their mode of subsistence and living conditions in different environmental settings. As dental attrition has been found a useful indicator of individual's age-at-death (8,12-15), various methods have been suggested to estimate the probable age of an individual using different attrition patterns and its stages. Kim et al., (16) suggested '0' to '8' stages of scoring system to calculate the age from dental attrition of premolar and molar teeth of an adult individual but dental conditions like tooth caries, fractures, and missing teeth were not considered for the purpose. Scott (17) proposed a simple '0-10' scale of dental attrition for molar teeth for age estimation of Amerindian skeletal samples. Miles (14) used the dental ages of juvenile skeletons exhumed from archaeological sites to provide the age-group specific dental attrition pattern to be used for a specific archaeological population, whereas Bayesian version of Miles' method was, later on, proposed to overcome its uncertainties and errors.

Forensic odontologists are often consulted for age estimation of the unknown deceased and the living individuals usually in cases related to adoptions, migrations, juvenile abuses, legal consent, asylum procedures and solving some other medico-legal cases like rape, marriage, kidnapping, job recruitment and social benefits like old-age pensions (18). Various morphological, radiological, chemical and radiological dental age estimation methods have been suggested for ageing the children and adolescents (19-20). Age estimated from the dental developmental and regressive changes is considered comparatively more reliable than the skeletal age; particularly for victim identification of mass-disasters. Age assessment in children and adolescents based on developmental changes in teeth is relatively more accurate than the age ranges estimated from the regressive changes in teeth. So, dental age estimation techniques are taken to be highly reliable in children but less accurate in the adults (21).

Dental eruption pattern (22-23), tooth mineralization status (19,24), cemental annulations or incremental lines (25-26), open root apices (27-28) are the commonly used methods for dental age approximations in children. After when the dental growth and maturation is completed, numerous external and internal factors affect the permanent teeth to introduce discrepancies between the chronological and dental age estimates (29), so age assessment is more difficult in adults and, there is no consensus on the most appropriate technique. Pulp-tooth area ratio (30), dental attrition or wear pattern (1-2), tooth color changes (31), secondary dentine deposition (32), periodontal recession or periodontosis (33), cementum apposition (34), root resorption and dentin translucency (35-36), volumetric and area measurement of pulp chamber and tooth surface (37-39), aspartic acid racemization (40-42) etc., are the commonly used dental age estimation methods for ageing adult skeletal remains found in forensic or bioarchaeological contexts.

Though dental attrition has been widely used as important indicator of the diet, age, and lifestyle of past as well as modern human populations by bioarchaeologists and physical anthropologists (43-44), it has received less attention for forensic age estimations. Dental attrition levels have been commonly used as a corroborative mean for aging the bioarchaeological materials (7, 12). Bioarchaeologically, dental attrition is supposed to occur at a specific linear rate in ancient samples to reflect the adult lifetime of an individual. Miles (14) assumed that rate of the tooth attrition remains same throughout adult life; provided dietary conditions remain the same. As attrition is not only age-dependent, it is also affected by the nutritional and dietary habits of a person, so it alone cannot be taken as a reliable indicator of age and thus, can corroborate age estimation from other techniques only.

The average stage of attrition (ASA) method has been proposed on the basis of average attrition stages of all the cusps present on the occlusal surface of molar tooth (1-2) and it is comparatively a newer and advanced method of dental age estimations. Each molar cusp is given a score and the averages of all four cusps are taken as ASA value. In order to find a relationship between the attrition stages and the age of an individual, the ASA value is calculated by classifying the attrition levels into 'nine' different stages depending upon the extent to which cusps are attrited; starting from 'no attrition' to the seriously 'attrited teeth'. The eighth and ninth stages of attrition are estimated from the attrition condition of the entire occlusal surface of the tooth. Separate regression equations have been suggested for each tooth type/number of both maxillary as well as the mandibular jaw. As the eruption pattern of the third molar is highly variable (45-46), the ASA method has been proposed on the basis of attrition stages of first and second molars only; separately for the upper and lower jaw.

Li and Ji (1) were the first researchers who applied ASA method for age-at-death estimation of 633 molars collected from the known 57 cadavers and 54 modern dry skulls of northeast China and found this method accurate enough for forensic purposes. The six linear regression equations proposed by Li and Ji (1) served as new graduation standard for age estimation based on dental attrition, rather than subjective analysis of attrition condition of the teeth recovered from forensic or bio-archaeological contexts. The maximum error of estimate from these regression equations was found to be 4.53 years. Ajmal et al (2) applied the ASA method on 100 molar teeth of known South Indians from Mangaluru (India) and formulated separate regression equations for individuals of the Indian population. The standard error of estimate (SEE) was suggested to be 2.7 to 3.9 years; lesser than the original age estimates proposed by Li and Ji (1).

The present study was undertaken to apply the previously published regression equations proposed by Li and Ji (1) and Ajmal et al., (2) for probable age estimation of teeth of unknown biological identity on the basis of attrition stages of teeth of known ages. The main objective was to have a rough age estimates of unknown Ajnala skeletal remains in order to corroborate or negate other forensic anthropological observations of these remains. The present study is an attempt to test the applicability and suitability of age estimation regression equations proposed by Ajmal et al (2) and Li and Ji (1) for age estimation of Ajnala skeletal remains on the basis of attrition stages of some randomly selected mandibular molars.

Brief historical account of Ajnala Skeletal remains

In April 2014, thousands of bones, teeth and items of contextual identity (like coins, medals, beaded arm-bands, metal rings, gold necklaces, stone bullets etc.) were excavated non-scientifically from an abandoned well found situated underneath a religious structure in a

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North Indian suburb of Ajnala (Amritsar, India) (Figure 1).

Figure 1 Excavated Ajnala Well.

Figure 2 (a): Badly damaged and commingled human remains.

Figure 3 (b): Heaps of badly damaged and commingled cranial and infra-cranial elements.

The written records mention that 282 Indian-origin revolutionary soldiers of the British army were killed whose dead bodies were disposed of in the said abandoned well (mass burial) in August 1857 (3-4). A religious structure was erected upon the periphery of the well, after putting charcoal and lime into it and raising a high mound of earth over into it. It was alleged in the book that the mutinied soldiers had murdered the British army commanders before fleeing away from the 'Mian-Meer' cantonment area (now in Pakistan). However, 282 fugitive soldiers were captured, imprisoned and murdered by the colonial rulers very next day under the administrative directions of author of the said book who was administrative head of Ajnala town at that time. The sanitary concerns and highly volatile socio-political scenario of the country were cited as the most feasible reasons for their immediate mass burial in the well. It was only in early 2014 that an Indian scholar of Amritsar (a city near Ajnala) happened to visit London History Museum who incidentally read the stated book. The possible availability of human cadavers of multiple individuals underneath the religious structure predominately worshiped by the local dominant community raised much media hype. Neither central nor state govt. authorities relied upon the media coverage or the written records suspecting it another hoax like Unnao treasure case. A saint from Unnao (Uttar Pradesh, India) had forecasted that huge amount of gold was available underneath a specific old temple which turned out to be false when Archaeological Survey of India (ASI) excavated the site in early 2014 (47).

The widespread media publicity and the consequent public outrage forced some local amateur archaeologists to dismantle the religious structure and relocate it to a newly constructed adjoining building. To scrutinize the truthfulness and authenticity of the written records were cited as the most immediate reason for this hurriedly carried out non-scientific exhumation of human remains from the well. The remains were found in the reported spatial sequence in the well sediments and the excavation continued for 4-5 days consecutively (48). It was only when the remains were completely exhumed by the curiosity seekers and then the author (JS) was warranted to take custody of the remains and the site by the concerned state government and asked for establishing the biological identity of the remains retrieved from the said well. On the first visit to the site, the author found that heaps of badly damaged and commingled (with numerous

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contextual or personalized items) human remains were scattered haphazardly near the site and very few of them were packed in big wooden boxes (Figure 2, figure 3). The forensic anthropology team took more than a week to sort out the teeth, jaw fragments, intact bones, contextual items of personal identity etc., from the human skeletal debris at the site. The dislocated teeth (out of socket ones), some jaw fragments (Figure 4) (having teeth in-situ, still fitted in jaw sockets), bones like femur, clavicle, talus, calcaneus, metacarpals, metatarsals, intact craniums and vertebrae were sorted out of the badly commingled human osseous remains. The well preserved skulls, bones, jaw fragments and teeth were transported to the author´s laboratory for their scientific analyses. No articulated skeleton or pelvis could be retrieved from the skeletal assemblage due to non-involvement of any osteological expert in the excavation process executed by amateur archaeologists. Presently, all the human remains along with contextual items retrieved from well assemblage (Figure 5) are housed in the author´s laboratory. Some amateur historians doubt the authenticity of the written versions of the book and argue that these remains allegedly belonged to the victims of Indo-Pak partition conflicts of 1947 when India got independence from British rule and the country was divided into two nations i.e., India and Pakistan ref.
The preliminary anthropological and radiological examinations, analyses of contextual items and the preliminary molecular findings have revealed that the Ajnala skeletal remains could belong to male adults reportedly killed in 1857 (48-49). The sex of remains was assessed from overall size and robustness of skulls, femur heads, clavicles, prominent development of occipital protuberance (inion hook), brow ridges and confirmed by preliminary mtDNA analyses (48). Ancient DNA extracted from 30 randomly selected teeth revealed that the skeletal remains belonged to adult North Indian males. The average age of the majority of recruits was found 30.3 years as estimated from radiological examination of pulp-tooth area ratio (PTR) of molars (49). In the present study, the author has estimated the probable age of Ajnala remains from the dental attrition. The null hypothesis was that the Ajnala skeletal remains belong to adult individuals as mentioned in the books (3-4).

Figure 4 Jaw fragments with teeth intact in sockets.

Figure 5 Contextual items retrieved from skeletal assemblage.

Material and Methods

Three hundred eighty-nine (N=389) intact mandibular molars (176 first and 213 second) were considered for age estimations on the basis of their attrition levels. A tooth does not need to be separated from the alveolus for scoring of dental attrition (50), so the in-situ mandibular molars (still in jaw sockets) were selected for the present study. The mandibular jaw fragments, having all molars intact into sockets, were selected from the skeletal assemblage excavated from an abandoned well found situated underneath a religious structure at Ajnala, Amritsar (India). Attrition stage was categorized from the wear condition of occlusal surface and the exposure of dentine or pulp chamber of the tooth (Figure 6 a-d) (51). The dental attrition wear level was identified using Smith (52) criterion and the attrition degree of all the four cusps of each molar was assessed on the basis of ten stage criteria proposed by Li and Ji (1). Then the average of all the four cusps was taken as ASA (average stage of attrition) value of attrition status of the particular tooth. The tooth having distinctly sharp cusps with well-demarcated ridges and gullies was designated to be healthy (stage '0') and the teeth with exposed dentine on entire occlusal surface was scored with the highest degree of attrition (stage '9'). Other stages were the variants of these two extreme stages of minimum and maximum attrition. Teeth with restorative work or grossly damaged occlusal

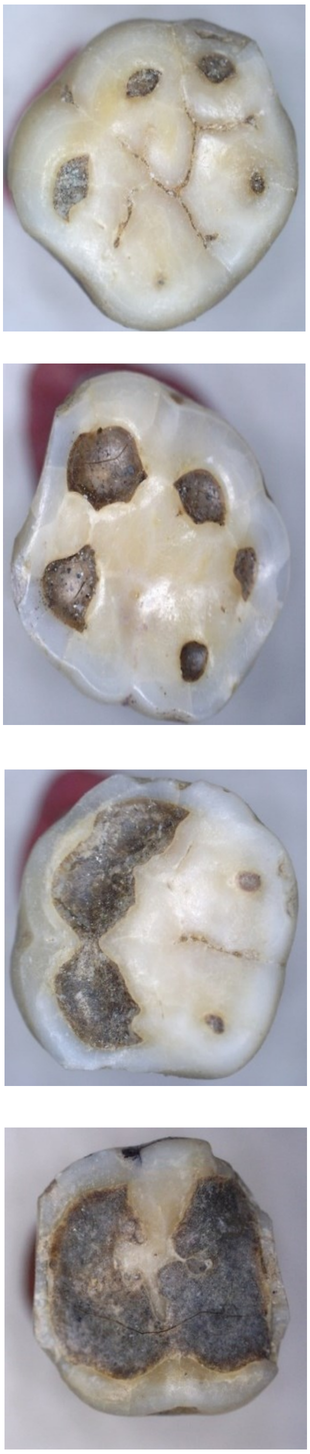
surface were excluded from the present study. To estimate intra- and inter-observer errors in scoring attrition stages, twenty-five molars were again examined again by the same author about one month, and they were also given to an independent odontologist/dentist for estimating the attrition stage. The weight Kappa score varied from 0.823 to 0.836 for inter- and intra-observation errors, showing a good strength of agreements between different observers and times of observations by the same observer.
The calculated ASA value of both first and second mandibular molars was put in the regression equations proposed by Li and Ji (1) and Ajmal et al., (2) for age estimation of Chinese and Indian subjects, respectively. The estimated age of molars was classified into five age-groups of ≤ 20 years, >20 - <30 years, >30 - <40 years, >40 - <50 years and >50 years, and the number and percentage of teeth in each group was also calculated. One sample Kolmogorov-Smirnov and the Mann-Whitney statistical tests were applied to test the significance of differences in age estimates from Ajmal et al., (2) and Li and Ji (1) regression equations given below:

Teeth type	Ajmal et al., (2) (Indians)	Li and Ji (1) Chinese
M1	24.58+3.78M1	12.76+6.30M1
M2	22.16+4.26M2	15.31+7.27M2
Both	20.08+2.46M1+2.15 M2	13.63+3.98M1+2.83 M2

Results

It is evident from the Table 1 that about 78 % Ajnala first molars fall in the age-group of 20-50 years when the ASA (average attrition stage) values of first mandibular molar were inserted in the regression equations proposed by Ajmal et al (2) for Indians and a small percentage (13.6%) belonging to the age-group below 20 years and few were above 50 (8.5%) years of age. The percentage of individuals classified to the age-group of 20-50 years increased to 89% when average attrition values of second molars were put into the respective regression formulae proposed by Ajmal et al (2). When the attrition stages of both first and second molars were used, it was found that 85.5% of teeth were in the age-group of 20-50 years. Thus, it may be concluded that 78 to 89 percentage molars were identified to the age-group of 20-50 years. A close correlation has been reported between the age and the degree of attrition of first molar (earlier erupting molars) which has a more advanced type of wear than the second or third molar.
When the regression equations proposed by Li and Ji (1) were solved with ASA values of Ajnala molars, it was found that none of the molar teeth was aged equal to or less than 20 years of age (Table 1). Also, a comparatively lesser number of individuals (5.68% from 1st molar and 2.34% from 2nd molar) were aged above 50 years of age than that estimated with Ajmal et al., (2) method. From average attrition stages of M1, M2 and both M1 and M2; 94.3 %, 97.7% and, 99.1% subjects were identified to the age-group of 20-50 years, respectively. Thus, we can say that age estimates from Li and Ji (1) regression equations endorsed the historical accounts that Ajnala skeletal remains belong to adult individuals.
The average age of the Ajnala individuals was calculated out to be around 34.5 years from Ajmal et al., (2) regression equations when used for M1, M2 or both the molars, with the standard deviation of 6.7 (approx.), variance of 45.0 and the coefficient of variation about 19.5 (Table 2). From Li and Ji (1), regression equations, the average age was estimated to be 33.6 years, though with higher values of coefficient of variation (32.66), standard deviation (11) and variance (122.0). Thus, it may be observed that age estimation from Ajmal et al., (2) regression equations is comparatively more reliable and practically applicable for forensic dental age estimations. The second molar provided almost similar age estimates by using both the regression equations.
Table 3 and 4 show the results of Mann-Whitney Test for differences in age estimates from two studies and one-sample Kolmogorov-Smirnov (K-S) Test. The former test is a non-parametric test to compare two population sample means and is an alternative to the independent t-test. One-sample K-S test is used to test the equality of continuous, one-dimensional probability distributions or to compare a sample with a reference probability distribution. It is evident from Table 3 that there are significant differences in age estimates from regression models of two studies for both first as well as second molar ($p<0.001$). The test distribution was found normal and mean age estimate from attrition stages of both 1st and 2nd molars was found to be approximately 32 years; though it was estimated as 37±6.91 years and 33.68±7.05 years from M1 and M2, respectively from regression model of Indian study (2) and 33.58±11.52 years and 34.97±12.04 years, respectively from regression model of Chinese study (1). The differences in

age estimates from one-sample KS test based on two studies were also found statistically significant (p<0.0001).



A

B

C

D

Figure 6 Different stages of dental attrition wear in Ajnala teeth samples (51).

Discussion

Human hard tissues like bones and teeth are unique biological materials to store crucial evidentiary information about past life events and the circumstances that happened around the time of death of an individual. The sequence of important life-events gets interlocked or imprinted within the human biological tissues of teeth and bones in different forms. Decoding the forensic bioarchaeological information contained within the dry bones and teeth pose a serious challenge for the anthropologists. Human evolutionary history and forensic osteological investigations would have remained inconclusive if these osseous remains had not been preserved to unravel their lifetime fate or happenings (53). The objective analysis of human skeletal remains can provide significant leads towards understanding the biological information encoded within bones and teeth. Forensic anthropological techniques are continuously evolving and expanding to interpret the hidden information within the skeletal and dental remains to reveal their identity affiliations.

Teeth often survive almost all post-depositional alterations in a better way than any other human tissue, so they generally play significant role in reconstructions of biological identity of badly damaged human remains. Dental attrition frequencies are reportedly higher in the archaeological skeletal materials than the dentition of modern industrialized populations. It may be probably due to the fact that individuals of ancient populations consumed comparatively rougher food items (containing more grits and larger grains); in addition to some non-masticatory like occupational or cultural uses of teeth by them (7, 54-55). Thus attrition wear patterns may play a significant role in age-at-death estimations in conjugation with other ageing methods. Dental attrition is a physiological change that progressively usually increases with advancing age (7-11, 56), and such wear changes can be quantified by using a variety of parameters/indices to adjunct other forensic odontological and morphological dental age estimation methods from teeth like pulp-tooth area ratio, aspartic acid racemization, age-dependent elemental variations etc., (57-58). Sexual variations have been reported in the occurrence of attritions in teeth of the two sexes, being more in males than the females (16, 59). The exertion of strong masticatory forces and consumption of rougher foods by males causes higher chances of dental attritions in males than the females (60-61). As Ajnala skeletal remains reportedly belonged to male sepoys of an army battalion (3-4) and, were badly damaged and discourageously commingled due to non-scientific excavation; no sex-dependent or jaw-specific differences in the attrition wear rates could be assesses in present study. Also, estimation of sex of Ajnala teeth was not objective of present study.

Mays (7-8) suggested that there exists a strong reliable correlation between attrition and adult age-at-death. However, some authors have argued that dental attrition is an individualistic feature (depending on lifestyle, nutrition, health, habits) and is not reliable enough for forensic dental age estimations, though it may contribute for bioarchaeological reconstructions. It has also been argued that age estimates from attrition quantified in one population cannot be applied for age estimation of other populations. Therefore, the suitability of such methods like ASA (average stage of attrition) should be tested for other populations (1).

As the entire excavation was carried out by amateur archaeologists without involving experts of any related scientific discipline, the remains were found badly damaged and commingled. Only teeth have been found well preserved to be used for establishing their identity affiliations. The shape, size, and morphology of different skeletal elements revealed that they probably belonged to adult males. The items of personal identity like coins and medals (having indented with Queen Victoria’s photograph and year of their make) further showed that the remains were thrown into the well around 1857 as none of them have inscriptions 1856 (Figure 4).

In the present study, 78% teeth were identified to the individuals whose age lie between 20 to 50 years from ASA regression equations of Indian individuals. These estimates correspond to the written records that the victims were adult individuals (3-4). As the attrition wear patterns appraised in one population cannot be used to assess age in other populations, so the average age estimates calculated from regression equations of Ajmal et al., (2) were considered valid and reliable. The coefficient of variation, standard deviation, and variance of age estimates were also comparatively much higher from Chinese regression equations (1) than the Indian formulae (2). Earlier studies have found that the ASA method is the suitable method for Indian subjects and has great forensic value as estimates are more accurate, reliable and reproducible. Li and Ji (2) reported that the ASA method provides the most accurate age estimates only if attrition stages are correctly quantified. The low degree of intra-and inter-observer errors in scoring attrition stages recorded in the present study suggested that attrition evaluation was not influenced by subjective assessments. As sexual differences in attrition levels were not possible to assess in present study, the ASA method (1) was chosen for age estimation of present study material. Though gender affiliations of Ajnala remains are doubtful, historically and radiographically the remains supposedly belonged to adult young males. The standard deviations of age estimates from two regression model suggested that 1st molars were more reliable than the 2nd molar from Ajmal et al., (2) equations than the Li and Ji (1) regression models (Table 4). The accuracy of results and ease in its use and understanding made ASA method as a golden standard for forensic age estimation of teeth belonging to Ajnala skeletal remains. The estimated dental age from ASA correlated with the age estimated by other odontological methods and it was in coherence with results of some previous similar studies (62). This is, the first Indian study which has utilized and compared the previously suggested regression equations for age estimation of an unknown sample and has proved useful in corroborating the age estimation from other scientific methods applied for this purpose.

Tooth attrition is a complex and multifactorial process being affected by a multitude of cultural, biological, physical, and chemical factors (44, 63). Though attrition based dental age estimation is a relatively simple, inexpensive and non-invasive method, it is the multifactorial causation of dental attrition that has lowered its efficacy for forensic age estimations. It has been suggested that molar age attrition techniques should be applied only if their application is constrained to the individuals of a particular cultural period and nutritional status. Age estimation on the basis of occlusal wear attrition patterns or levels may not be the as precise to be accepted for forensic purposes as attrition process has been found dependent on various factors such as dietary habits, mastication, number of teeth present, presence or absence of opposing teeth, presence of artificial teeth, geographic and environmental factors. Dental attritions may not provide the precise estimates of chronological age of a person (63-65); the rough age estimates (with wide confidence intervals and ranges) can be a useful tool for ageing the bioarchaeological teeth samples (7 12, 14, 56,61, 66). Present study observations may not be useful for age estimations of Ajnala dental remains, but may support other methods used for ageing Ajnala skeletal remains.

Conclusion

Tooth attrition is a complex process being affected by a multitude of factors. It occurs at a specific linear rate throughout life and is age-dependent. Dietary conditions can affect its progression. Attrition alone cannot be taken as a reliable age indicator in bioarchaeological contexts but can support other methods of age estimation In the present study, the regression model developed by Ajmal et al2 from average stages of attrition of molars of Indian subjects of known age were applied to estimate the age of unknown Ajnala skeletal remains. It was estimated that about 80% Ajnala mandibular molars belonged to adult individuals aged between 20-50 years and which is in close coherence with written records that adult army recruits killed by colonial rulers were dumped into an abandoned well at Ajnala (Amritsar, India). Though the ASA method is not reliable for age estimation in forensic investigation, present study results may provide deep insights into bioarchaeological affiliations of these remains when studied with the application of other latest advanced scientific techniques like computer-assisted assessment of attrition stages (67), mtDNA dataset analyses or stable isotope analyses.

Conflict of interest statement

There is no conflict of interests with anyone regarding the publication of this brief communication. Ethical clearance has been obtained from Institutional Ethics Committee of Panjab University, Chandigarh (India) vide letter no. PUEIC/2018/99/A/09/01 dated: 28.01.2018 for carrying out research on the material used in present study.

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Table 1 Number and percentage of teeth classified into different age-groups using two regression equations based on average stages of attrition (ASA) suggested by Ajmal et al (2) for Indians and Li and Ji (1) for Chinese individuals.

Method	Tooth type	No. and %	≤ 20	>20 - <30	>30 - <40	>40 - <50	>50
Ajmal et al (2)	M1 (N=176)	n	24	55	49	33	15
		%	13.64	31.25	27.84	18.75	8.52
	M2 (N=213)	n	0	91	72	27	23
		%	0	42.72	33.80	12.68	10.80
	Both M1& M2 (N=213)	n	20	74	80	28	11
		%	9.39	34.74	37.56	13.15	5.16
Li and Ji (1)	M1 (N=176)	n	0	27	96	43	10
		%	0	15.34	54.55	24.43	5.68
	M2 (N=213)	n	0	79	104	25	5
		%	0	37.09	48.83	11.74	2.34
	Both M1& M2 (N=213)	n	0	78	111	22	2
		%	0	36.62	52.11	10.33	0.94

Table 2 Descriptive statistics of age estimates from regressions models of two studies.

Method used	Teeth type	No. of teeth	Min Age	Max	Mean Age	Std. Deviation	CV	Variance	Skewness	Kurtosis
Ajmal et al (2)	M1_	176	28.36	58.6	37.07	6.91	18.65	47.82	0.702	0.050
	M2_	213	26.42	60.5	33.68	7.05	20.94	49.76	1.420	2.204
	Both	213	22.23	56.2	32.61	6.15	18.87	37.86	0.605	0.539
Li and Ji (1)	M1_	176	19.06	69.46	33.58	11.52	34.32	132.83	0.702	0.050
	M2_	213	22.58	80.74	34.97	12.04	34.42	144.91	1.420	2.204
	Both	213	16.46	67.85	32.15	9.40	29.24	88.40	0.563	0.371

Table 3 Mann-Whitney U Test for differences in age estimates from Ajmal et al (2001) and Li & Ji (1995).

Tooth type	Authors	N	Median	η1 - η2	95.0 Percent of η1 - η2	Test of η1 = η2 vs η1 ≠ η2
M1	Ajmal et al (2)	176	35.920	4.575	(2.685,6.466)	0.0001**
	Li & Ji (1)		31.660			
M2	Ajmal et al (2)	213	32.810	0.390	(-1.428,2.022)	0.05117*
	Li & Ji (1)		33.485			

Table 4 One-Sample Kolmogorov-Smirnov Test.

Tooth type →		Average age estimated from Ajmal et al (2)			Average age estimated from Li & Ji (1)		
		M1	M2	Both	M1	M2	Both
No. of teeth →		176	213	389	176	213	389
Normal Parameters[a,b]	Mean	37.0744	33.6800	32.6129	33.5840	34.9697	32.1532
	Std. Deviation	6.91498	7.05375	6.15276	11.52497	12.03773	9.40200
Most Extreme Differences	Absolute	0.119	0.152	0.046	0.119	0.152	0.050
	Positive	0.119	0.136	0.046	0.119	0.136	0.050
	Negative	-0.104	-0.152	-0.046	-0.104	-0.152	-0.048
Test Statistic		0.119	0.152	0.046	0.119	0.152	0.050
Asymp. Sig. (2-tailed)		0.000[c]	0.000[c]	0.200[c,d]	0.000[c]	0.000[c]	0.200[c,d]

a. Test distribution is Normal, b. Calculated from data, c. Lilliefors Significance Correction, d. This is a lower bound of the true significance