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CASE REPORT Open Access

Identification of fragmented cranial remains excavated from a site adjoining Ajnala well: a forensic anthropological case report

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Abstract

Background Mankind have suffered several unexpected disasters, heinous crimes, and brutalities, resulting into large number of unknown skeletal remains to be identified and repatriated to their relatives.

Case presentation One such incident of mass killing of 282 Indian-origin soldiers by the colonial rulers in 1857 was reported from Ajnala (India) in early 2014. The damaged cranial fragments belonging to two individuals were retrieved from the vicinity of Ajnala mass-burial site later in 2014. In present study, the retrieved cranial elements were examined forensic anthropologically to assess whether their affinity is akin to the slain Ajnala sepoys or to the deceased who were buried prior to or later than it. The morphological and molecular analyses of these remains affirmed their affiliation to the mutiny sepoys of 1857 killed at Ajnala.

Conclusions The forensic anthropological and molecular examinations of cranial fragments found that these remains belonged to the individuals killed in the Ajnala incident. The multidisciplinary approach applied for identification of the badly damaged, fragmented, and commingled human remains helped in their presumptive identification.

Keywords Forensic anthropology, Unknown human remains, Ajnala, Fragmented cranial elements, Case study

Background

Forensic anthropological identification of commingled human remains recovered from diverse contexts is a challenging but complex task due to material obstacles like poor preservation, severe damage or fragmentation, missing elements and, lastly the complexities and inadequate records about the past armed conflicts, and mass disasters producing such human remains. Restoring individual identity to such remains and their repatriation to their relatives/friends for final closure comfort is the ultimate purpose of almost all forensic anthropological identifications (Wagner, 2019). The exhumation of intact skeletal remains from the burial sites like trenches, abandoned wells, and nallahs, using expert-mediated retrieval techniques is essentially required for successful identification strategies of unknown human remains. Identifying commingled human remains requires significant time, energy, resources, expertise, and application of innovative methods. The recovery, inventory preparation, segregation, and articulation of commingled human remains from mass burial sites is considered more tedious than the individual graves. DNA analysis of skeletal remains is an effective and highly appropriate method used for identification of the victims of the armed conflicts these days (Lisman et al. 2023). A multidisciplinary approach (osteometric pair-matchings, exclusions and associations, simulations, DNA sequencing strategies) is direly needed to resolve the issue of identification of the commingled assemblages in forensic anthropology (Ubelaker et al. 2019). Each commingled assemblage is unique in its

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history, challenges, and context, and no single identification approach is universally applicable to the diverse situations under which commingled human remains are recovered (Palmiotto et al. 2019). Integrated inputs from archaeology, modern genetics, and ancient DNA has made it possible to study prehistoric events in reality and thus, hold promise for the reconstruction of the human past (Horsburgh and McCoy 2017). Stable isotope analysis of human hard tissues (bones and teeth) can provide important information about the geographical origin and dietary preferences of the deceased or missing individuals in medico-legal scenarios (Kootker et al. 2023). As far as present study skeletal material is concerned, the commingling was further exacerbated by their non-scientific excavation by the amateur archaeologists, which further discouraged the identification efforts of these cranial fragments, thus preventing reconstruction of nature of violence committed with the victims.

Case presentation

The written records about the availability of human remains dating back to 1857 lying buried in an abandoned well underneath a religious structure at Ajnala (Amritsar, India) (Fig. 1) instigated the conscience of certain locals to excavate the site (Sehrawat et al. 2016). A very disappointing and self-congratulatory account of killing 282 sepoys of 26th Native Bengal Infantry regiment was found reported in some contemporary literary texts (Cooper 1858, East India Papers 1859, Bates and Carter 2017). The uprising (known as the first Indian War of Independence) against colonial rulers started with the revolt of the aggrieved native Hindu and Muslim Indian soldiers, known as sepoys, against the colonial occupation of Indian territory by the British East India Company over fears that new gun cartridges were greased with animal (beef and pork) fat strictly forbidden by their respective religious faith. It is mentioned that the slain sepoys had revolted against the orders of their British commanders, killed few of them and fled from the Mian Meer cantonment (now in Pakistan); however, the fatigued and bullied sepoys were captured and imprisoned after few days of their revolt. The very next day, the dishonoured sepoys were called forth (ten by ten) to face the inevitable death from the sullen firing of volley of distant musketry firings. The corpses were dumped into the said abandoned well, citing sanitary concerns and contemporary socio-political scenario of the country as the most compelling reasons for their immediate mass burial in the said disused well. A religious structure was erected upon the periphery of the well, after putting charcoal and lime into it.

Fig. 1 Excavated Ajnala Well

It was only in early 2014 that the revelations by written records instigated some amateur local archaeologists to dismantle the religious structure and relocate it to an adjoining newly constructed structure. To scrutinize the truthfulness of the facts mentioned in the written records was reported as the most immediate reason for this hurriedly carried out non-scientific exhumation of human remains from of this mass burial site at Ajnala. The excavation lasted for 4–5 days continuously and the remains were recovered from the well in same spatial sequence as reported in the book. Neither the federal nor the state government authorities relied upon the written records and the media coverage about the availability of any such remains beneath a religious structure. The bones were found badly fragmented (consequent upon non-scientific excavation) and were also taphonomically modified due to their prolonged outdoor exposure to sun, wind, rain, and variations in temperature or humid conditions (Lloveras et al. 2018). Stone bullet injuries in the frontal and temporal regions of few intact skulls recovered from the skeletal assemblage corroborated the historians' view of barbaric treatments of the victims or their skulls (Sehrawat and Sankhyan, 2020). Thus, non-scientific excavation resulted into heaps of badly damaged, fragmented and commingled remains, thus posing a serious challenge for the identification pursuits. The possibility of assigning identity and regional origin to these badly damaged unknown human remains is a compelling effort and potentially significant conclusions can be drawn from their scientific analyses using different methodologies.

A big soil mound housing the fragmented cranial fragments (of possibly two individuals) was located in the

close vicinity Ajnala well during a landscaping activity by the locals. When the soil mounds were removed *as a whole* and were soaked into big water container, it yielded craniofacial elements of two crania, few vertebrae and metatarsals, a silver coin and some concrete pieces (Figs. 2 and 3). To shed light on the population affinity of these two cranial fragments and find any association with the reported Ajnala skeletal assemblage retrieved from the well, a series of bio-archaeological investigations like osteological analyses, ancient DNA analysis, stable isotope dietary reconstruction, and paleopathological reconstructions were carried out. Present study was carried out to ascertain whether the recovered cranial fragments were synchronous to Ajnala skeletal remains or they belonged to a different traumatic situation.

Methodology

The fragments belonging to Specimen 1 and Specimen 2 were designated as JPN-1 and JPN-2, respectively for convenience of the researchers. All the skeletal and dental remains were cleaned of any extraneous dirt or soil and were examined for their morphological features to estimate their sex and age identity, traumatic or pathological lesions of each specimen separately, using forensic anthropological methods and techniques described in the literature. Standardized protocol was used for collagen extraction from the lower second molar of both the specimens which were sent to UC Davis Stable Isotope Facility (California) for further analysis/processing for stable isotope (carbon, nitrogen, oxygen) analysis; and the strontium isotope concentrations were measured at Department of Earth Sciences, Newfoundland University, Canada. The ancient DNA from the petrous bone of each specimen was extracted, amplified and sequenced at the state-of-the-art facility at Birbal Sahani Institute of Paleosciences (BSIP), Lucknow (India). The protocol used for the isolation and library preparation was same as in a previously published article (Sehrawat et al. 2022) with slide modifications (Dabney et al. 2013; Korlevic et al. 2015) and the extracts were converted into the libraries (Rohland et al. 2015). The enriched libraries were then sequenced by Illumina NovaSeq instrument using paired end 180 bp

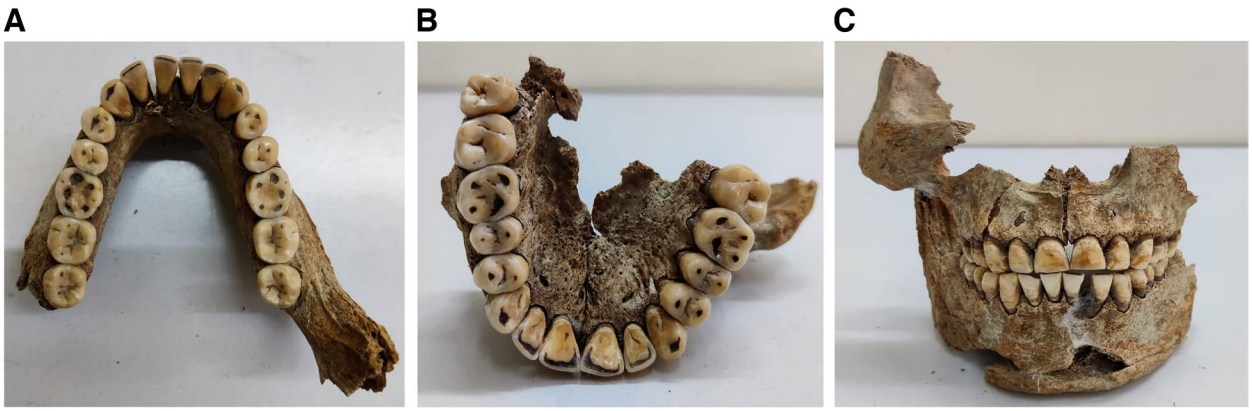

Fig. 2 **a** Mandible of specimen I (JPN-1). **b** Maxilla of specimen I (JPN-1). **c** Jaw fragments put together of specimen I (JPN-1)

Fig. 3 **a** Mandible of specimen II (JPN-2). **b** Maxilla of specimen II (JPN-2). **c** Craniofacial fragments of specimen II (JPN-2)

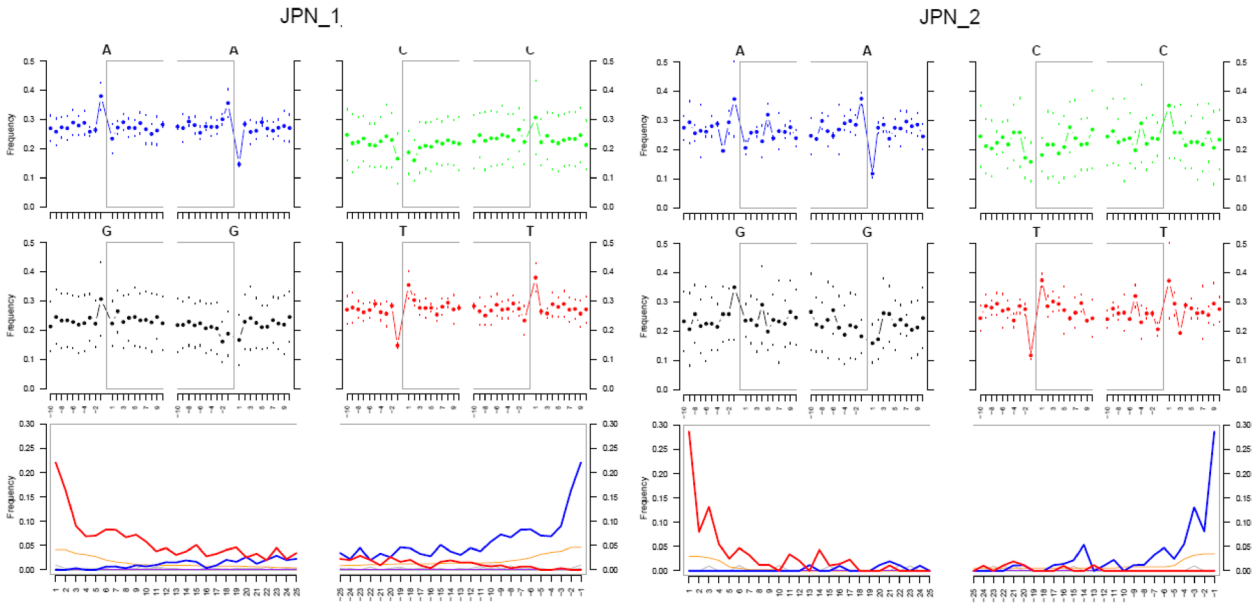

Fig. 4 Showing the map DNA damage pattern in JPN-1 and JPN-2 samples. The orange line in the that there is slight 0.05% of soft clipping, demonstrating the reason of low coverage

reads. Further analysis was performed by in-house pipeline with both 'rcrs' mitochondrial and 'hg19' reference genome (Behar et al. 2012; Li and Durbin 2011). By using mapDamage, DNA damage pattern was found in both the specimens which further confirmed their authenticity (Fig. 4A) (Jónsson et al. 2013). In the mitochondrial genome analysis, the coverage of JPN-1 was upto 3×, while it was very low (0.02×) in JPN-2 (Fig. 5). Hence, JPN_2 was excluded from further analyses and major analyses were focussed on JPN-1 only.

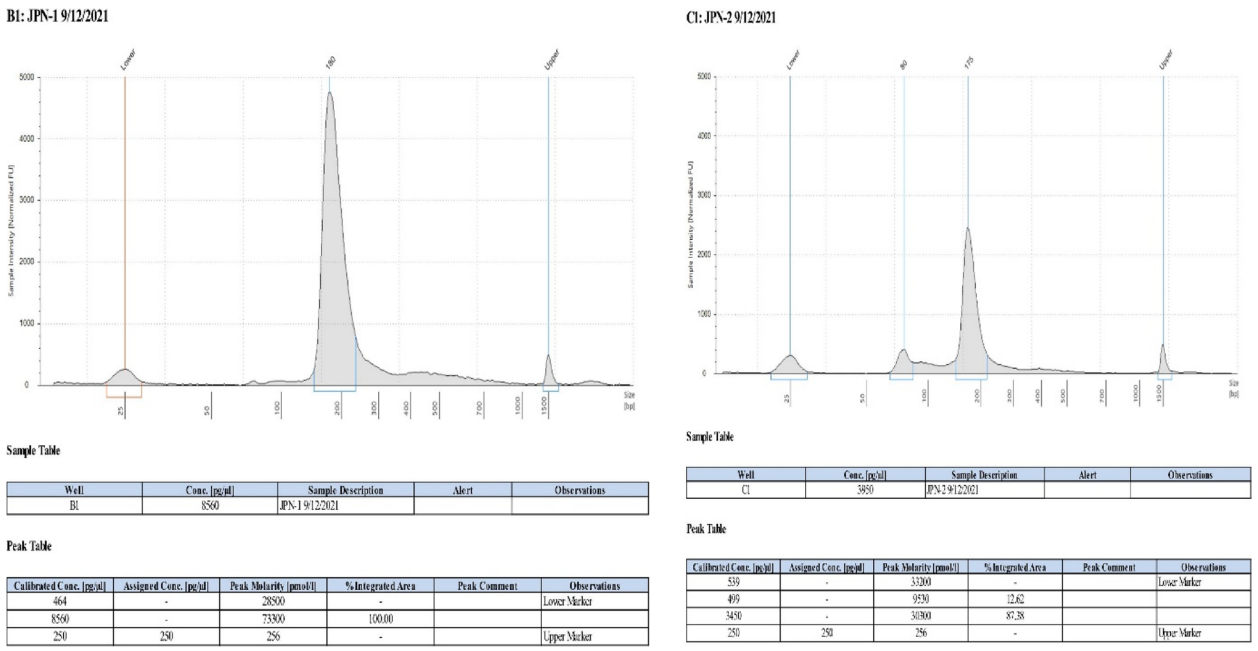

Fig. 5 DNA sequence peaks of sample JPN_1 and JPN_2

Results

Forensic anthropological observations

When the skeletal remains recovered from the two separate locations in the fields adjoining Ajnala well were articulated anatomically, the remains were found distinctly belonging to two different individuals; so they were classified arbitrarily as JPN-1 and JPN-2 for convenience purposes only:

Specimen I (JPN-1)

The biological sex was estimated from the general robusticity of the skull fragments, the size and shape of the gonial angle, prominence of mental eminence, and the meaty mastoid process which revealed it supposedly belonging to a male. The eruption of third molars with relatively minimal wear of the teeth, and apparent degree of cranial suture fusion (although this may be affected through taphonomic damage/distortion) indicated the fragmented remains were of a young-mature adult, though other skeletal indicators may put this specimen in older age category. Estimation of biological age merely from dental wear becomes a bit uncertain, still the standard tooth wear profiles can be used to have reasonable age estimates. The occlusal surface wear analysis showed that the teeth belonged to a pretty youthful individual, but possibility of using a soft diet by this individual cannot be ruled out. The pelvic pubic symphysis or auricular surfaces were not available in the retrieved skeletal assemblage for ascertaining the biological age of the specimen. Cusp number, size and morphology can be valuable tools for understanding who a person is. This individual appears to have a cusp 7, on the lower left first molar, and cusp 6 on lower left third molar. Furthermore, the accounted date of these remains fall entirely outside forensic significance, so a bioarchaeological evaluation would be more than sufficient.

Specimen II (JPN-2)

The morphological examination of teeth and jaw bone was used for forensic anthropological examination of this specimen. Excessive bruxism was absent; lower molars were having modest fanning. Abrasions at first molar cusps; absent or very minor or not-measurable abrasion on second and third molars. No periodontal disease indication. The individual looks belonging to a grown up male who was suffering from gum problem. The occlusal attrition reflects chewing gritty and/or hard to chew foods or rough food items. The mental eminence (damaged?) and submental depth loosely suggest the individual to be male. All molars are fully erupted and have a good deal of wear, so individual to be adult but not young. A mesial notch in the right second mandibular incisor might be a pipe groove or a non-alimentary facet. The anterior part of left side of the mandible looks like having a periodontal pocket on, but that could be staining too. The worn dentition was probably caused by acid erosion rather than the abrasive diet. Bulimia can result in erosion of dental enamel, but not like this which appears to be present on occlusal surfaces only. The acid erosion from frequent vomiting is present particularly on all lingual surface enamel. Acidic erosion from any locally consumed contemporary drink (something very acidic in nature) has been found present on all tooth surfaces (labial, lingual, occlusal) of this specimen.

Ancient DNA and stable isotope analyses of specimens (JPN-I and JPN-2)

The haplogroup of JPN-1 DNA was determined using haplogrep 2.0 (Weissensteiner et al. 2016), which confirmed that JPN-1 skeletal and dental fragments particularly were of a male, belonging to 'M-52' haplogroup with 63.15% confidence. These results were in accordance with the observations reported in the previously published article (Sehrawat et al. 2022), where the geographical affiliation have been shown to be non-local to the site and belonging to Eastern Uttar Pradesh, Bihar and some North-eastern Indian. The whole genome analysis results disappointed the authors as the coverage was very low to conclude any type of results in terms of autosomes, i.e., 0.02× and 0.01× for JPN-1 and JPN-2 respectively (Figs. 4 and 5).

Stable isotope values of carbon, nitrogen, oxygen and strontium were measured from second mandibular molars of both the samples; the values have been presented in Table 1. The C: N ratio was 3.27 and 3.28, respectively for JPN-1 and JPN-2 specimens, respectively. Though no valid conclusions can be drawn from the isotopic concentrations of two samples only, the values indicate their strong association with Ajnala skeletal remains regarding their dietary status and geographic affinity, i.e., these two individuals were too non-local to the site (Sehrawat et al. 2020, 2022; Sehrawat and Rai 2023).

Discussions

Individualization of unknown human remains is one of the primary objectives of forensic anthropological casework in medicolegal and humanitarian contexts

Table 1 Stable isotope values of the samples JPN_1 and JPN_2

Stable isotope	JPN_1	JPN_2
Carbon $\delta^{13}C$ (VPDB)	−18.36	−17.14
Nitrogen $\delta^{15}N$Air (‰)	7.22	8.78
Oxygen $\delta^{18}O$VSMOW (‰)	19.40	18.98
Strontium	0.722668	0.718462

(Wagner, 2019). The issue of ownership, identification and repatriation of unknown human remains to their next kin is very complex and sensitive and stands as the ultimate purpose of the forensic anthropologists. The resolution of any forensic puzzle necessities analysis from different perspectives and with multiple but complementary tools. The knowledge limitations and associated errors generally jeopardize the traditional identification efforts due to mistakenly identified remains and the inadvertent effects of commingling. Whether the deceased are civilian victims of violent conflicts or combatants who sacrificed on behalf of a nation, the efforts to identify the commingled ones are predicated on the insistence that names count and bodies belong. Recovering, reassembling, and naming individuals who died as a result of war or violent conflict are themselves a form of honouring and remembering. Numerous studies have reported been reported in the literature where forensic anthropological and molecular techniques helped in identification of challenged human remains found in such contexts (Grisbaum and Ubelaker 2001, Komar 2003, Spradley 2014, Lisman et al. 2023).

In such an instance, the skull fragments retrieved from a soil mound adjoining Ajnala mass assemblage site raised some forensic archaeological queries to be answered from their forensic anthropological examinations. As the amalgamation of physical and molecular anthropological methods can significantly contribute in interpretation of biological profile such badly fragmented human remains (Pilli et al. 2023), the collective approach of morphological and mtDNA analysis was used to infer the biogeographical origin of the retrieved skull fragments via DNA analysis.

The morphological cranial trait analysis of both the specimens revealed that both the individuals were adult males, thus human cranial fragments can provide significant information about the biological identity of unknown human remains, even when metric or molecular evaluations are not possible due to poor preservation, incompleteness, or fragmentation of such remains (Pink 2016; Mann and Hunt 2019). A unique combination of cranial traits may have supportive role in presumptive identification of human crania (Palamenghi et al. 2021). The mitochondrial DNA (mtDNA) analysis of the challenged human remains is widely used to establish forensic identity of damaged human remains found in different contexts (Sehrawat et al. 2022). The isotopic signatures of both the specimens showed their closeness to previously estimated values of C, N, O, and Sr from teeth samples of Ajnala skeletal remains (though isotopic values only two samples do not qualify for drawing conclusions). Though the use of stable isotopes for provenance of unidentified human remains is mainly restricted to osteoarcaheology, it is getting more uptake in forensic archaeology and medico-legal death investigations, albeit with limited accuracies (Bartelink et al. 2016; Ubelaker and Francescutti 2020).

The physical evidences in the form of skeletal or dental remains, along with items of contextual identity can demonstrate identity of the victim/s. No such items of personalized identity were recovered with the fragmented Ajnala cranial elements. The badly damaged and fragmented nature of the cranial fragments prevented investigators to perform their standard skeletal analysis for identification purposes (Sorg and Haglund, 2002) and the profile specific complete bones were found missing from the assemblage retrieved from the soil mounds. The morphoscopical, osteological, molecular and stable isotope results supported the versions that cranial fragments of two individuals excavated from an adjoining site to Ajnala well belong to the same group of individuals who were apprehended and killed at Ajnala and the cadavers were dumped into an ancient abandoned well. It may be possible that cadavers of these individuals might have been buried after the main mass burial site (Ajnala well) was filled with sand. Present case report endorsed that individuals buried in shallow burial near Ajnala well belonged to slain sepoys who fled from Mian Meer cantonment. Identifying the victims of historical wrongdoings like war or war-related crimes or violent conflict, natural disasters, or whatever other incidents of mass fatality, is relatively a modern phenomenon. The increased scientific efforts about more focussed application of technological armentorium towards individuated post-mortem identification have attributed social value to the forensic identification of the dead. How many people are buried in a mass grave, or which bones belong to which body, and how the individuals ended up as a collective or in a common burial site are the major forensic queries to be responded by forensic anthropologists.

Conclusions

The anthropological, molecular, and stable isotope analysis of the cranial elements retrieved from the fields adjoining the Ajnala well found that these fragments belong to two victims of the same incident of Ajnala massacre of sepoys whose remains were dumped into the said abandoned well. These cranial elements might belong to the left-over victims whose bodies were removed from a small setting near Ajnala police station where 45 individuals died due to suffocation. Thus, the application of multitude of techniques can help in identification of badly damaged and commingled human remains found in forensic contexts.

Abbreviations

UC	University of California
BSIP	Birbal Sahani Institute of Paleosciences
mtDNA	Mitochondrial DNA
C	Carbon
N	Nitrogen
O	Oxygen
JPN	Named arbitrarily to represent 1st names of persons involved in analysis

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Authors' contributions
JSS conceptualized the manuscript, did forensic anthropological examination of the material uses, took photographs, wrote original draft of the manuscript, and provided final inputs in the manuscript; NR carried out DNA extraction and analysis of the samples, provided figures of DNA analyses, and reviewed the final draft of the manuscript.

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Availability of data and materials
Data and material used in present manuscript are available with the corresponding author and can be obtained via email, when needed.

Declarations

Ethics approval and consent to participate
Necessary permissions have been obtained from Institutional Ethics Committee to carry out research work with Ajnala skeletal remains. The ethical approval for carrying out research on the skeletal and dental material used in present study was obtained from the Panjab University Institutional Ethics Committee (PUIEC) of Panjab University, Chandigarh (India) vide letter no. PUIEC/2016/41/20//05 dated: 18.8. 2016 and PUEIC/2018/99/A/09/01 dated: 28.01.2018.

Consent for publication
Both the authors have consented to submit this work to Egyptian Journal of Forensic Sciences for consideration of publication of this work.
All authors declare that the manuscript is not currently under submission to any other journal.

Competing interests
The authors declare that they have no competing interests.

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