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Stable C and N isotope analysis of human dental remains retrieved from an ancient well at Ajnala (Punjab, India): Assessment of Dietary status and geographic affiliations

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Research Article

Keywords: Ajnala skeletal remains, dental collagen, stable δ13C & δ15N isotopes, dietary status and geographic affiliations

Posted Date: November 17th, 2021

DOI: https://doi.org/10.21203/rs.3.rs-1053629/v1

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Abstract

Stable isotope analysis of biogenic tissues like tooth and bone has become a widely recognized and increasingly important method for provenance of human remains, particularly in bio-archaeological and forensic investigations. Establishing the dietary status and identity of unknown human skeletal remains retrieved from forensic anthropological contexts is a challenging task. Thousands of unknown human osseous remains along with the personalized contextual items, reportedly belonging to 282 Indian soldiers killed in 1857, were excavated non-scientifically from an abandoned well at Ajnala (Amritsar, India). In present study, the isotopic concentrations of carbon (C) and nitrogen (N) were estimated from the dentine collagen extracted from 21 first molars to provide information about the geographic affinity and dietary status of the individuals killed in Ajnala. As diagenesis affects bone more severely than enamel or dentine due to increased porosity of the former, so teeth were preferred to bones for stable isotope analysis in present study. The literature supported C: N range of 2.8-3.6 was considered as cut-off for the well-preserved collagen and the samples with values outside this range were considered to be altered or contaminated with non-collagenous materials. The interpretation of the obtained isotope values from Ajnala teeth samples indicated the consumption of C_3/C_4 mixed diet (though some samples showing marine diet) by the victims which supported the previous observations about the dietary status of Ajnala victims estimated from prevalence of various dental pathologies. Though C and N isotopes are generally not the best indicators of geographic origin, they can be used to for the purpose only if they show different dietary inputs of C3 and C4 plants. Present study results provided scientific confirmation to the written historical accounts that Ajnala skeletal remains belonged to the individuals belonging to the Indian states of Awadh (north-eastern Uttar Pradesh), Bihar and Bengal and some norteastern states.

1. Introduction

The establishment of identity and geographic affiliations of badly damaged and commingled human skeletal remains excavated from an abandoned ancient well at Ajnala (Amritsar, India) has presented a challenging scientific task for the investigators (Sehrawat et al, 2016). Thousands of human remains along-with numerous contextual items and artefacts were unearthed in early 2014 from a disused well located underneath a religious structure (Figure 1). The non-scientific excavation carried out by the local amateur archaeologists resulted into serious damage and fragmentation of already fragile and commingled human remains (Figure 2). The retrieved Ajnala skeletal remains were handed-over to the first and corresponding author by the concerned govt. authority (as the sole overseer of these remains) for their biological profiling purposes vide reference letter no. DCAM/2015/842-845 dated: 11/02/2015. Various hypotheses regarding these remains were put forward and one such hypothesis maintains that the remains are related to the victims of sepoy mutiny of 1857during India's 1st freedom struggle war against the colonial rulers in India. The dictated use of beef and pork greased cartridges by the British commanders hurt the religious sentiments of the Indian soldiers of 26th Native Bengal Infantry regiment wherein sepoys from Eastern UP, Bengal, Bihar, coastal Orissa and some norteastern states were reportedly recruited as army sepoys (Cooper, 1858; East India Papers 1859, Bates and Carter, 2017).

(Figure 3). This ignited the buried rebellion against the rule of British East India Company which was initiated by ‘Mian Meer’ cantonment sepoys stationed at Sialkot near Lahore (now in Pakistan) and spread widely to other cantonments. The remains exhumed from Ajnala well (referred to as Ajnala remains) are believed to belong to 282 soldiers belonging to 26th Native Bengal Infantry battalion who had reportedly murdered their British commanders and fled from the cantonment. Various written accounts mention the capture of the fugitive soldiers near the banks of river Ravi and their eventual killings by the British loyal forces (Cooper, 1858; East India Papers 1859, Bates and Carter, 2017). The cadavers were stated to be dumped in the reported disused well nearby to the killing site, citing the existent socio-political situation of the country. Some locals believed that the said human remains belong to the individuals killed in August 1947 during pre-independence Hindu-Muslim conflicts. This theory was based on the proximity of Ajnala to the India-Pakistan border and the recurrent Hindu-Muslim clashes witnessed in 1947 leading to the death of thousands of the civilians. However, the indentations of year of make of on the recovered coins and military medals and the radiocarbon dating results of few dental collagen samples collected from Ajnala skeletal assemblage refuted the later theory (Sehrawat et al., 2016; Sehrawat et al., 2020). The authors have undertaken the task of establishing the origin of these remains (local versus non-local and their geographic affinity) and the present study is one such endeavour to understand the scientific basis of the 1st origin hypothesis about these remains.

One of the aspects in tracing the provenance of human remains scientifically is establishing their dietary habits. The agriculture and animal husbandry advancements, development of urbanized societies and commercial trade practices have progressively increased the extent of dietary foods (Schmitt et al., 2012); however, factors related to preservation, recovery or storage pose some complications in the elucidation of dietary compositions of archaeological skeletal collections. Stable isotope analysis of human remains has proved to be a powerful tool for revealing the dietary habits and the food sources consumed by the individuals in the past (Richards, 2020). Stable carbon and nitrogen isotope analysis of bone collagen is a well-established method for the direct dietary reconstruction of past populations. Stable carbon isotope values (δ13C) are used to differentiate dietary carbon sources. In most archaeological contexts, carbon isotope patterning allows us to identify the use of plants of different photosynthetic pathways (mainly C3 and C4 plants), as well as to distinguish between terrestrial and marine-based resources (Sealy, 2005). Stable nitrogen isotope values (d15N) primarily reveal trophic level effects in the food chain. As fractionation of δ15N is a stepwise process, there is generally a 3–4‰ enrichment per trophic level (Hedges and Reynard, 2007). The stable carbon and nitrogen isotope composition of dental collagen samples have been widely investigated over the last several decades as a proxy for the proportions of different potential dietary components. The δ 13C values reflect the relative contributions of aquatic and/or terrestrial sources of carbon in the diet whereas the δ 15N values indicate both the protein source and trophic level of an individual before their death (Bird et al., 2020). In order to determine diet-based geographical localities of the Ajnala victims, the C and N stable isotope analyses were carried out.

For stable isotope analysis and interpretation, essential geographical data was also acquired for the region under investigation. Present day Ajnala town (31.84 N; 74.76 E) is situated in northeast to the district headquarters of Amritsar (Punjab, India), having an average elevation of 213 meters from the

mean sea level. The semiarid climate is prevalent here and this region typically experiences four seasons primarily: winter season (December to March, when temperatures can drop to −1°C, summer season (April to June, when temperatures can reach up to 45°C or more), monsoon season (July to September) and post-monsoon season (October to November). According to Indian Meteorological Department, Pune (https://mausam.imd.gov.in/), the annual rainfall here is about 703 millimetres. This area is covered with fluvial quaternary sediments derived from the Himalayan regions. The soil comprises of good sedimentary porous unconfined aquifers having the rich groundwater level. Thus, wells in the ancient times were constructed as a source of drinking as well as irrigation water for the local people.

2. Material And Methods

2.1 Material:

Twenty-One (N=21) intact 1st molars collected from the jaw fragments of the Ajnala skeletal assemblage were chosen for estimating the stable isotopic concentrations of carbon and nitrogen. All the selected first molars were obtained from the left mandibular fragments to prevent any chance of sample duplication from the same individual. The selected teeth were cleansed, rinsed and dried before drilling them for their dentine powder in the laboratory of first and corresponding author (JS). The collagen from the vialed dentine samples was extracted at the laboratory of senior-most author (NR) using Merck's Amicon Ultra 1.5 micro-filter columns (UFC903024, Merck KGaA, Darmstadt, Germany), before sending the encapsulated collagen samples to UC Davis Laboratory, California (US) for their stable isotope concentrations/values. As concentration of carbon and nitrogen in collagen is the most reliable indicator of collagen preservation (Ambrose, 1990), dentin collagen was preferred to bones for stable isotope analyses in present study due to the commingled and badly fragmented/damaged nature of the bones (likely to be contaminated) (Figure 2).

2.2 Methodology:

The crushed dentine powder samples (about ~0.5–1.0 g in weight) were stored in 15 ml centrifuge vials and then ~ 0.2 M HCl was added to the vialed samples and were left immersed for over 2 days at room temperature. After two days, the acid was decanted and vials were refilled again with 0.2 M HCl. The samples were stored for another two days at same temperature. This procedure was repeated until the collagen contents were almost demineralised. The nearly complete demineralised collagen samples were rinsed with deionised water (DI water) and NAOH for their neutralization from contamination of any humic acids etc. A mild acidic solution was added to the samples which were then placed on the heating block (Thermo Shaker) for gelatinization. Post-gelatinization, the samples were transferred to 30 k 24pk Amicon ultra filters (Merck, UFC903024) and were centrifuged at 2500 RPM for 15 to 25 minutes, to completely remove the base-soluble humic acids and other contaminants. Boudin et al. (2013) have stated that "ultrafiltration of bone/dentine collagen is an effective method for the removal of low molecular weight contaminants from bone/teeth collagen, though it does not remove high molecular weight contaminants, such as cross-linked humic-collagen complexes" (Higham et al. 2015). After these

processes, the samples were piped out to micro tube vials and frozen at −40°C and afterwards lyophilised overnight.

C: N ratios were calculated using the equation:

C: N= %C/%Nx14.007/12.011

The literature supported C: N range of 2.8-3.6 was considered as cut-off for the well-preserved collagen and only samples that met these collagen quality criteria were included in this study; the samples having values outside this range were considered to be altered or contaminated with non-collagenous materials. The prepared collagen samples were tested out by elemental analyses having C/N ratios which ranged near around ~2.8 to 3.6 for all 21 samples, which is the indication of good collagen yield and purity of samples from contaminations (Higham et al. 2015). The concentrations of C and N isotopes (as C: N ratio) in collagen is the most reliable criteria for determining the preservation status of the collagen. Teeth have comparatively significant lower collagen concentrations, lower carbon and nitrogen concentrations in collagen than the bone, though have similar C: N ratios. Carbon and nitrogen concentrations and C: N ratios are relatively constant over a wide range of collagen concentrations (Ambrose et al 1990). Further, these samples were packed into tin boats and processed to be sent to the UC Davis Laboratory, California by the first and corresponding author for the measurements of their C and N stable isotopic values.

3. Results And Discussions

The carbon and nitrogen stable isotope signatures can provide insights into geographic affinity and dietary habits. The δ13C values of collagen and carbonate apatite are typically used to track the type of plant food consumed by herbivores (Bocherens and Drucker, 2013). The nitrogen isotopic signature of a given individual depends on the isotopic signature at the base of the food web to which it belongs, and on the position of the specimen within the food web. Different plants may use nitrogen in different proportions, thus leading to varying isotopic fractionation and δ15N values.

The stable isotopic results of carbon and nitrogen ($\delta^{13}C$ & $\delta^{15}N$) have inferred that Ajnala victims were dependent upon a large range of diets as indicated by the stable isotope values which ranged from −18.7‰ to −22.9‰ and +7.6‰ to +11.7‰ with an average of −20.49±1.2‰ and +9.31±1.1‰, respectively (Table 1). All reported carbon and nitrogen isotope values are averaged values based on duplicate analysis and are reported in ‘permil’ (‰) and were calibrated to VPDB and AIR, respectively, using USGS40 and USGS41 (Coplen, 2011). Purely terrestrial diets show the stable carbon isotope values, $\delta^{13}C$ = −21.5‰ (Palincaș, 2017). Since the diet by tradition varies geographically, and is based on different primary products derived from plants with different 13C content, the amounts in tissues will vary accordingly (Schoeller et al 1986). Figure 4 shows the average and standard deviations of stable carbon and nitrogen isotopic results of dentine collagen with their C/N ratios

Table 1
Stable carbon and nitrogen values of human dentine collagen samples (N=21)

S. No.	Sample ID	IRMS Lab Code	Sample type	$\delta^{13}C_{VPDB}$ (‰)	$\delta^{15}N_{Air}$ (‰)	TC%	TN%	C/N Ratio
1	AJ 83 B	1659954	Dentine Collagen	-18.7	9.1	39.1	11.4	3.4
2	AJ 08 B	1659913	Dentine Collagen	-18.7	9.0	44.8	13.6	3.3
3	AJ-4	8457	Dentine Collagen	-18.9	9.4	40.7	14.4	2.8
4	AJ-2	8454	Dentine Collagen	-19.1	9.7	40.9	13.3	3.1
5	AJ 77 B	1659951	Dentine Collagen	-19.2	7.6	41.2	12	3.4
6	AJ-25	8549	Dentine Collagen	-19.4	9.8	28.3	9.5	3.0
7	AJ-8	8469	Dentine Collagen	-19.5	10.3	40.8	12.7	3.2
8	AJ 74 B	1659949	Dentine Collagen	-19.8	9.3	43.8	13.2	3.3
9	AJ-3	8455	Dentine Collagen	-20.4	9.0	39.4	10.8	3.6
10	AJ-7	8468	Dentine Collagen	-20.7	8.4	41.7	11.5	3.6
11	AJ 67 B	1659947	Dentine Collagen	-20.8	8.4	44.4	12.7	3.5
12	AJ 04 B	1659911	Dentine Collagen	-20.9	8.2	43	12.2	3.5
13	AJ 89 B	1659960	Dentine Collagen	-21.0	8.1	41.5	11.7	3.6
14	AJ 79 B	1659952	Dentine Collagen	-21.0	9.6	42.4	12	3.5
15	AJ 94 B	1659964	Dentine Collagen	-21.1	9.8	42.2	11.7	3.6
16	AJ-12	8473	Dentine Collagen	-21.3	11.4	40.8	11.3	3.6
17	AJ-15	8484	Dentine Collagen	-21.3	11.7	40.7	12.4	3.3

S. No.	Sample ID	IRMS Lab Code	Sample type	$\delta^{13}C_{VPDB}$ (‰)	$\delta^{15}N_{Air}$ (‰)	TC%	TN%	C/N Ratio
18	AJ 62 B	1659940	Dentine Collagen	-21.6	8.8	44.7	12.4	3.6
19	AJ-5	8458	Dentine Collagen	-21.7	8.1	39.5	11	3.6
20	AJ-24	8548	Dentine Collagen	-22.4	9.4	29.1	8.7	3.3
21	AJ-13	8474	Dentine Collagen	-22.9	10.5	38.7	11.1	3.5
			Average	**-20.49**	**9.31**	**40.37**	**11.89**	**3.4**
			Std. dev.	**1.2**	**1.1**	**4.3**	**1.3**	**0.2**

Generally, teeth are considered as better choice for geochemical investigations since they are the hardest and most calcified structures of the human body; able to resist all sorts of putrefactions, destructions, degradations, and survive the detrimental impacts of fire, environmental pollutants, and taphonomic damages etc. The carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) isotope ratios in dentine collagen are very informative about the dietary intakes of people, primarily the protein intake. Nowadays, people living in large urban areas have easy access to a wide range of food products derived from a broad geographic range termed as “global supermarket diets”. Whereas, in rural and isolated regions, particularly in developing countries (like India), a significant portion of dietary items are usually derived from the locally available food sources; either self-produced or purchased from nearby localities. During the mid-19th century India (in context to the dating of remains used in present study), people were not used to migrate to other places for obtaining food items but remained dependent upon the locally available dietary resources. The stable-isotope analyses can be of considerable help in evaluation of the geographical localities of past populations on the basis of their osseous remains (bones and teeth). The collagen protein remains well protected and preserved for long time within the dentine and it remains insensitive to the minor changes in dietary components, so it can serve as the most ideal osseous tissue for scientific analyses about past lifetime events of an individual to indicate the signatures of major human food chains. The technique of stable isotope analysis has the advantage of providing long-term information about major dietary components of foods eaten by an individual (Hedges 2003; Sealy, 2001). Twenty-one collagen samples were extracted from the dentine and processed for measurement of their $\delta^{13}C$ and $\delta^{15}N$ values to assess the food habits, dietary patterns and geographic affinity of Ajnala skeletal remains in an attempt to authenticate or refute the written accounts whether these remains belonged local or non-local people and if the later, then to the reported geographic regions of India i.e., Bengal, Bihar, coastal Orissa, norteastern Indian states of Manipur, Meghalaya, Tripura, and Awadh (north-eastern Uttar Pradesh). Though a total of 58 collagen samples were processed for their stable isotopic concentrations, but 37 samples had isotopic concentrations beyond acceptable limits (see Supplementary Table), so they were discarded for further analyses and thus only 21 samples satisfied C/N ration within permissible limits.

Table 2 shows the carbon and nitrogen isotopic compositions of few main groups of food sources which are generally consumed by the human beings; and their respective enrichment in isotopic composition from one trophic level to the next higher level (Minagawa and Wada, 1984; Cheung et al., 2017; Britton et al., 2018). This approach of stable isotope analysis is largely based on the fact that plants photosynthesize via three pathways; the C_3 pathway (C_3 plants), C_4 pathway (C_4 plants) and CAM pathways (Crassulacean Acid Metabolism) when plants utilize either via C_3 or a C_4 photosynthetic pathway, depending on the prevailing environmental (wet or dry) conditions. Plants using different pathways have remarkable differences in their stable isotopic signatures; C_3 plants (Barley-wheat based crops) generally grow in higher latitudes having cool and wet climate and they have $\delta^{13}C$ values ranging from −20‰ to −35‰, with an average of −27‰. The C_4 plants include crops like maize, millet, and sorghum, which generally grow in latitudes having warm and humid climatic conditions and have $\delta^{13}C$ values ranging from −9‰ to −16‰, with the mode at about −12‰ (O'Leary, 1988; Farquhar et al., 1989; Cerling et al., 1997). Though the stable $\delta^{15}N$ is not a direct indicator of food or dietary sources because the $\delta^{15}N$ values of plant and animal sources are often influenced by the external mechanisms like aridity (Hartman 2011), soil type (Fiorentino et al. 2011), manuring (Bogaard et al. 2007) and water efficiency (Aguilera et al. 2008), however, when nitrogen ($\delta^{15}N$) values are considered with the carbon isotope values, a convincing information about the ecological conditions of the area can be reconstructed (Schoeninger et al., 1992). The analysis of several stable isotopes in teeth and hair can help to determine both the earlier origin and more recent residence of an unknown dead body and thus facilitate identification work (Alkass et al 2013). This might be explained by a more traditional diet with locally typical basic nutritional food sources during childhood than the diet during adolescence Rice is the most vulnerable C_3 crop whose stable $\delta^{13}C$ of value is approx. −24.14‰, whereas $\delta^{13}C$ in modern millet crops (C_4 based) is ranged from approx. −7.0‰ to −9.13‰ (Chen et al., 2017). These barley-wheat-rice crops are largely grown in the Indian states of Punjab, Haryana, Uttar Pradesh, Bihar and Bengal.

Table 2
The carbon and nitrogen isotopic compositions of few main groups of food sources generally consumed by the human beings and their respective enrichment in isotopic composition from one trophic level to the next higher level (Minagawa and Wada, 1984; Cheung et al., 2017; Britton et al., 2018)

Common Food Sources for human	Enrichment in δ13C ‰ due to Trophic level fractionation	Mean ($\delta^{13}C$ ± Std. dev.) ‰	Enrichment in δ15N ‰ due to Trophic level fractionation	Mean ($\delta^{15}N$ ± Std. dev.) ‰
C_3 plants	-27 to -20.0	-23.5±2.5	2.0 to 8.5	5.3±2.3
C_4 plants	-13.0 to -5.7	-9.4±2.6	3.0 to 9.5	6.3±2.3
C_3 herbivores	-22.0 to -16.5	-19.3±1.9	4.3 to 9.06	6.7±1.7
C_4 herbivores	-10.7 to -7.0	-8.9±1.3	5.1 to 11.46	8.3±2.2
C_3/C_4 mixed herbivores	-17.9 to -13.0	-15.5±1.7	6.2 to 11.96	9.1±2.0
Fin Fishes	-16.0 to -17.7	-16.9±0.6	11.1 to 15.9	13.5±1.7

The Indo-Gangatic Plain (IGP) is a great alluvial crescent stretching from the Indus River system in Pakistan to the Punjab Plain (in both Pakistan and India) and the Haryana Plains to the delta of the river Ganga in Bangladesh. It covers the states of Punjab, Haryana, Delhi, Uttarakhand, Uttar Pradesh, Bihar and West Bengal (Saud et al 2012). d13C and d15N values were found as -25.5 ± 0.5 and 9.6 ± 2.8 %, respectively for particulate matter of Delhi region. For Varanasi, the average values of d13C and d15N of PM10 were -25.4 ± 0.8 and 6.8 ± 2.4 %, respectively. The average concentrations of d13C and d15N were found -26.0 ± 0.4 and 7.4 ± 2.7 %, respectively over Kolkata with ranges of -26.6 to -24.9 % and 2.8 ± 11.5 %, respectively (Sharma et al 2015). The average value of d13C of PM10 over all locations was -25.6 ± 0.6 %. The exploitation of C and N isotopes from human osseous tissues (Bones and teeth) for assessing geographic affinity and dietary intakes is very limited. Present study results indicate that major portion of dietary protein of Ajnala's victims came from the terrestrial ecosystem which included mainly the C3/C4 mixed type of vegetation i.e., they were most probably dependent upon the barley-wheat-rice based crops (Figure 5). These types of crops/vegetation are extensively grown in the Uttar Pradesh, Bengal, Bihar and some northeaster states of India in the Gangetic plain which constitute the major staple foods for the inhabitants/natives of the area. Interestingly, for one or two individuals, perhaps the major portions of their dietary protein were coming from the marine sources as they showed enriched (about greater than 11‰) values of $\delta^{15}N$; they most probably belonged to the Bengal or coastal Orissa state regions as it is the natives of these regions who frequently use fishes in their meals rather than the other regions of India.

Six food sources most commonly used to occur in human diets, their (food source) mean $\delta^{13}C$ and $\delta^{15}N$ isotopic values of bone collagen with trophic level correction are given in Table 2. Furthermore, to provide more clarity and better understanding of stable isotopic datasets, all individuals were statistically arranged into three groups by 'ISOSOURCE version 1.3.1" software as presented Table 3 and shown in

Figure 5. The three included food sources are ‘C3 herbivores’, ‘C3/C4 mixed herbivores’ and ‘Fin fishes’ only on the basis of $\delta^{15}N$ isotopic values as $\delta^{13}C$ isotope were not considered for the purpose due to showing almost similar values. From Table 3, it can be evidently illustrated that almost all victims used to consume the C3/C4 mixed herbivores (goat/sheep are most likely in India); thus predominantly C3/C4 mixed food items. During the mentioned period of mid-19th century, the agricultural facilities in India were very limited and crops were mainly dependent on monsoonal rainfalls only. In present times, people of Gangetic plain generally cultivate both the C3 (Barley-wheat based) and C4 (Millet based) crops and; the same crop pattern might have been practised at that time too. In these regions of Gangetic plain, the seasonal rainfall occurs only for 2-4 months throughout the year and thus the region remained under drought conditions for most of the months of the year, thus, mixed type of vegetation (C3 and C4) is found grown in the Gangetic plain. As the states of Uttar Pradesh (only eastern), Bihar and Bengal regions cover about more than 70% of the area of Gangetic plain, therefore, C3/C4 mixed feeders probably belonged to these regions and people in and around Ajnala (site of excavation) are mostly dependent upon C3 plant foods (barley-wheat based crops). Since fish constitutes a staple/ very essential component of food of people of Bengal and Orissa, few victims having shown the signatures of fin fishes diet might have belonged to the Bengal or Orissa region. All these exercises of isotopic analyses of dentine collagen have, thus, almost confirmed the geographical identity of Ajnala skeletal remains reportedly belonging to slain soldiers of 26th Native Bengal battalion who were killed by the British loyal forces in 1857. The 26th Native Bengal Infantry battalion comprised of mixed individuals from reported Indian states/regions (Cooper 1858, Sehrawat et at 2020). The C: N data show a mixed C3/C4 diet, which is common in many regions of India, including Bengal, Bihar, Orissa, eastern Uttar Pradesh, Manipur, Meghalaya, and other coastal Indian states (Figure 3).

Table 3
Probable food sources of studied Ajnala samples classified into three major food sources/habits with the help of ‘ISOSOURCE model’ (version 1.3.1).

Food Sources	C_3 herbivores + Barley-Wheat-Rice Based crops	C_3/C_4 mixed herbivores + Barley-Wheat-Rice Based crops	Marine Fin Fishes + Barley-Rice-Wheat Based crops
Population (n)	00	21	00

The integration of present study stable isotope results with, their pathological status (Sehrawat et al., 2018), odontometrics (Sehrawat and Singh 2019), elemental profile (Sehrawat and Singh 2020a) and odontological aging (Sehrawat 2020; Sehrawat and Singh, 2020 b) have firmly supported the written accounts that the retrieved skeletal remains belonged to sepoys killed in 1857 who belonged to specific geographical regions of eastern and north-eastern Indian states.

4. Conclusions

Using stable carbon and nitrogen isotope analysis of 21 dentine collagen samples of Ajnala skeletal remains (historically belonging to sepoy-victims of sepoys mutiny of 1857), the geographical origin of the remains was corroborated. The $\delta^{13}C$ and $\delta^{15}N$ isotopic results showed the predominance of C3/C4 mixed diet, with some marine food consumers, expected from the mentioned states of Uttar Pradesh, Bihar, Bengal, coastal Orissa and some north-eastern regions as these types of food habits (geographically) are largely confined to the people of aforesaid states of India. Thus, the results of present scientific investigation/endeavour have largely authenticated the written historical version about the identity affiliations of Ajnala skeletal remains and discarded all other hearsay tales about them.

Declarations

Conflict of Interest:

There are no potential conflicts of interest for publication of this research work. The retrieved Ajnala skeletal remains were handed-over to the first author (JSS) by the appropriate authority as the sole overseer of these remains for their biological profiling purposes vide reference letter no. DCAM/2015/842-845 Dated: 11/02/2015.

Acknowledgements:

The first and corresponding author (JSS) is highly thankful to Science and Engineering Research Board (SERB), Department of Science and Technology (DST), Govt. of India for providing financial support for this work in the form of Core Research Project (Animal Sciences), Grant no. SERB/F/4790/2018-2019, which helped in conceptualization, experimentation and designing of present study. The senior-most and corresponding author (NR) is highly thankful to the Director, Birbal Sahni Institute of Paleosciences (BSIP), Lucknow for extending all available facilities to help in extraction and packaging of collagen from the powdered dentin samples before sending the processed samples to an overseas laboratory for stable isotope analyses. SD thanks the Director Wadia Institute of Himalayan Geology for providing necessary infrastructure facilities. The author (NR) was also helped by Mr. Nikhil Patel, Research Scholar and his mentor Dr. Rajesh Agnihotri, Scientist-F, Birbal Sahni Institute of Paleosciences, Lucknow, in encapsulation of the prepared collagen samples.

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Figures

Figure 1

Ajnala well before and after its excavation

Figure 2

Badly damaged and commingled nature of non-scientifically excavated human remains from Ajnala well

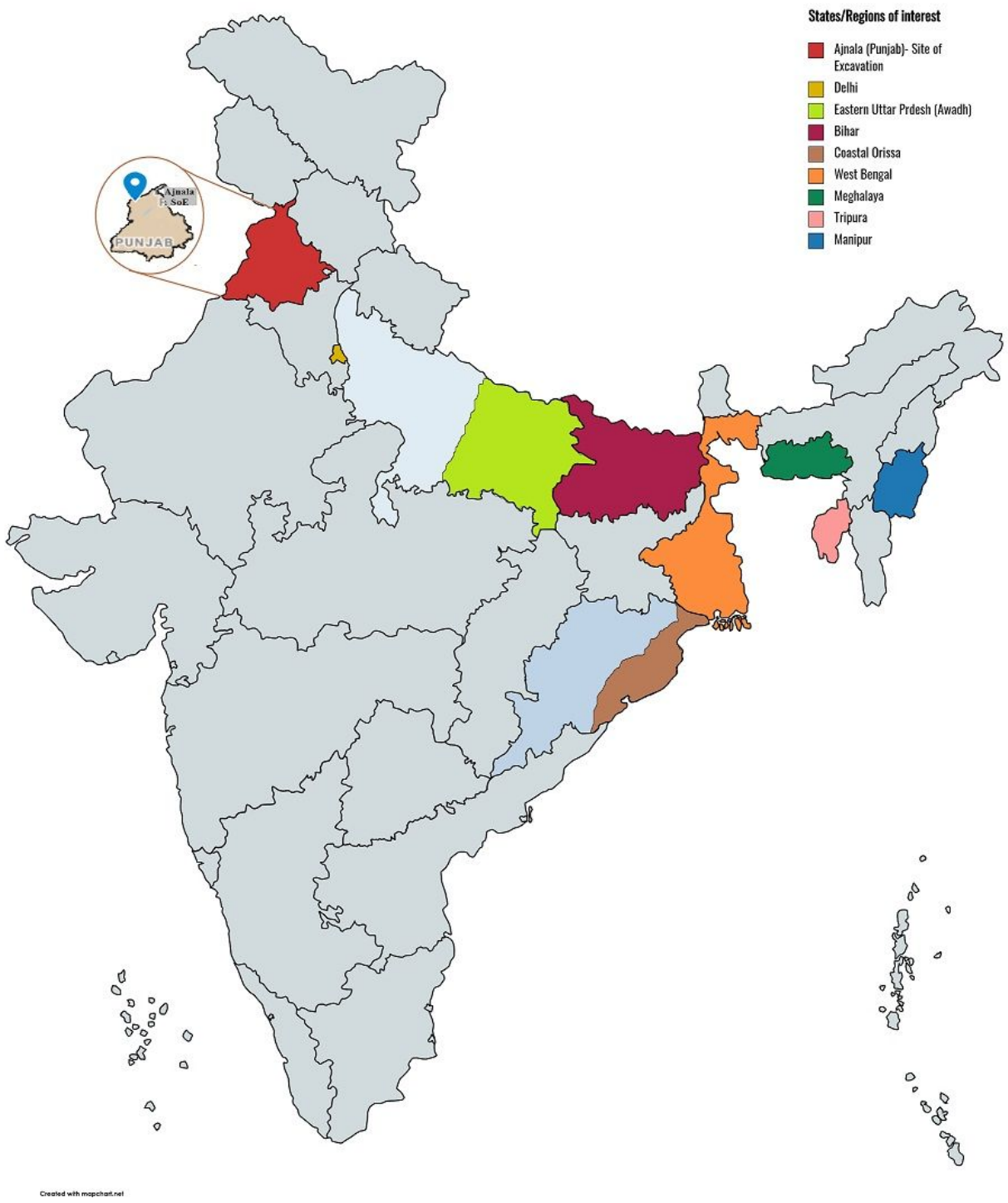

Figure 3

Cartographic representation of the site and reported states/regions of India to which the sepoys belonged

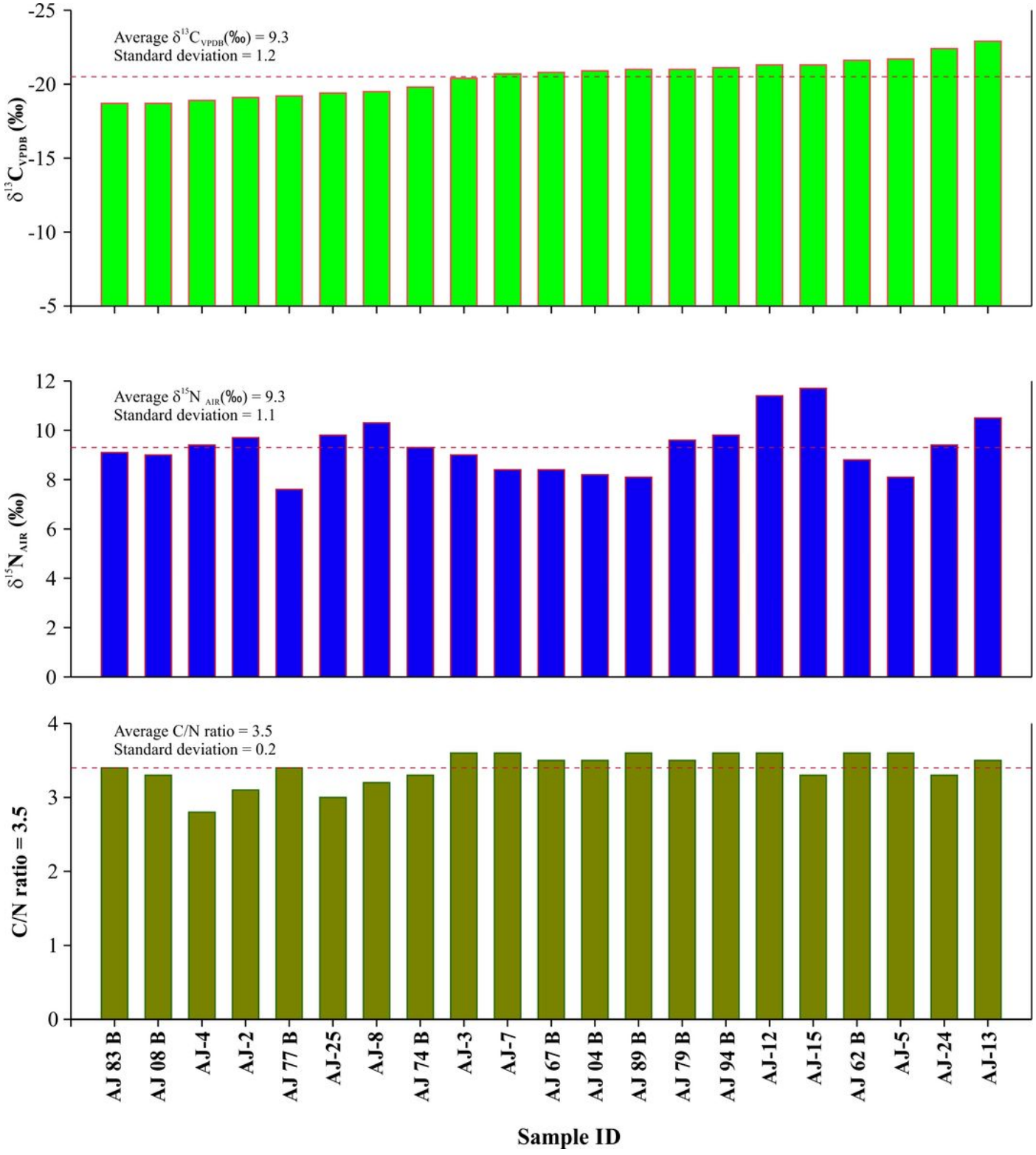

Figure 4

The average and standard deviations of stable carbon and nitrogen isotopic results of dentine collagen with C/N ratios.

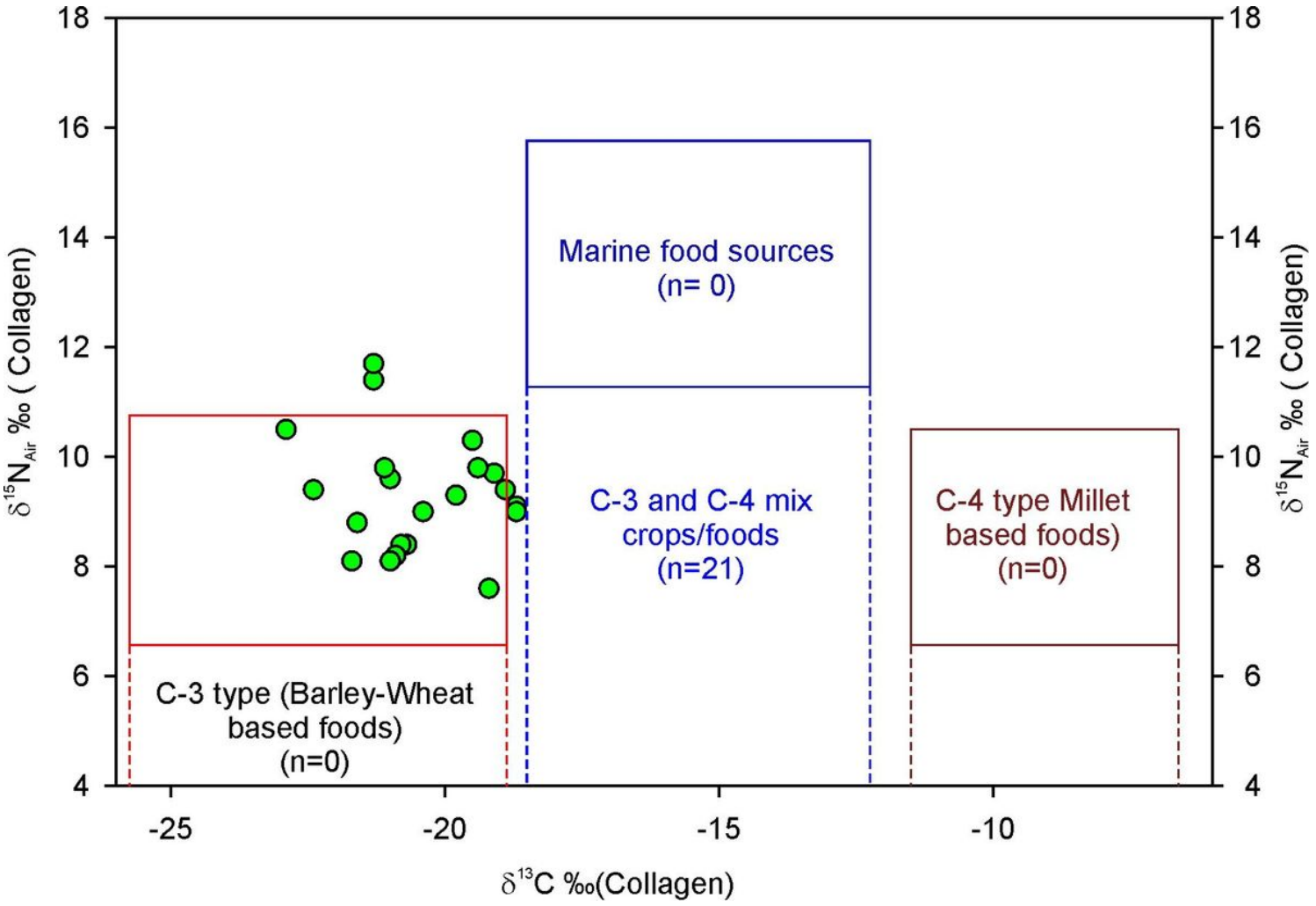

Figure 5

Food/dietary sources of Ajnala individuals estimated from ISOSOURCE version '1.3.1' software

Supplementary Files

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- SupplementaryTable.docx