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Role of stable isotope analyses in reconstructing past life-histories and the provenancing human skeletal remains: a review

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ABSTRACT: This article reviews the present scenario of use of stable isotopes (mainly $\delta^{13}\text{C}$, $\delta^{15}\text{N}$, $\delta^{18}\text{O}$, ^{87}Sr) to trace past life behaviours like breast feeding and weaning practices, the geographic origin, migration history, paleodiet and subsistence patterns of past populations from the chemical signatures of isotopes imprinted in human skeletal remains. This approach is based on the state that food-web isotopic signatures are seen in the human bones and teeth and such signatures can change parallelly with a variety of biogeochemical processes. By measuring $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ isotopic values of subadult tissues of different ages, the level of breast milk ingestion at particular ages and the components of the complementary foods can be assessed. Strontium and oxygen isotopic analyses have been used for determining the geographic origins and reconstructing the way of life of past populations as these isotopes can map the isotopic outline of the area from where the person acquired water and food during initial lifetime. The isotopic values of strontium and oxygen values are considered specific to geographical areas and serve as reliable chemical signatures of migration history of past human populations (local or non-local to the site). Previous isotopic studies show that the subsistence patterns of the past human populations underwent extensive changes from nomadic to complete agricultural dependence strategies. The carbon and nitrogen isotopic values of local fauna of any archaeological site can be used to elucidate the prominence of freshwater resources in the diet of the past human populations found near the site. More extensive research covering isotopic descriptions of various prehistoric, historic and modern populations is needed to explore the role of stable isotope analysis for provenancing human skeletal remains and assessing human migration patterns/routes, geographic origins, paleodiet and subsistence practices of past populations.

KEY WORDS: Stable isotope analysis, Bones and teeth, different isotopes, life-histories, provenance of skeletal remains, forensic anthropology

Background

An element contains the atoms of stable isotopes which have same number of protons but different number of neu-

trons and therefore, differ in their masses. The minor variations in atomic mass causes the stable isotopes of an element respond at different rates in chemical reactions (Bartelink et al. 2014). Gener-

ally, the lighter elements are in isotopic forms while the heavier elements are infrequently isotopic. Fry (2006) stated that lighter isotopes such as hydrogen (H), carbon (C), nitrogen (N), Oxygen (O), Sulphur (S) constitute between 95 and 99.6% of their natural abundance whereas heavier isotopes constitute between 1 and 5% only. Stable isotope ratio is usually described as the ratio of the heavy isotope to the light isotope such as $^{13}\text{C}/^{12}\text{C}$, $^{15}\text{N}/^{14}\text{N}$ in connection with a known standard. The stable isotope values are denoted in parts per thousand or permil (‰). U.S. National Bureau of Standards and the International Atomic Energy Agency (IAEA) have provided international laboratory standards for describing isotope values. The $\delta^{13}\text{C}$ isotopic values for most of the organisms are negative relative to the standard, whereas the $\delta^{15}\text{N}$ isotopic values are usually positive in organisms, it is because of the fact that atmospheric nitrogen is depleted more in ^{15}N than the most living things (Bartelink et al. 2014). McKinney et al (1950) proposed an equation to calculate the delta ratio from the solution of the $\delta(\text{‰}) = [(R_{\text{sample}}/R_{\text{standard}}) - 1] \times 1000$ (where $R = ^{15}\text{N}/^{14}\text{N}$ for nitrogen and $R = ^{13}\text{C}/^{12}\text{C}$ for carbon). Chemical methods investigate the chemical composition of bones or teeth for the reconstruction of paleodiet provide the most relevant information about the original contents of the diet. The organic phase of bone largely consists of collagen protein which can remain preserved for up to 70,000 years (Bocherens et al. 1994), whereas inorganic phase includes calcium hydroxyapatite. Appropriate chemical methods have been developed to analyse both these phases of bone to reflect the dietary components of an individual; the organic portion being more resistant to

contamination after burial than the inorganic phase.

The isotopic composition of the human skeleton remains like bone and teeth can give information about various aspects of past life. The isotopic analyses of bone and teeth help to reconstruct those human history events/processes which are difficult to reorient from traditional anthropological methods (Szostek et al. 2015). The stable isotope analyses has been found an established method for reconstructing infant feeding and weaning practices, paleodiet, geographic origin, migration routes, etc., from the human skeletal and dental remains (Fuller et al. 2006). The stable carbon and nitrogen isotopes have been widely used to reveal ancient human behaviours like food economy, differences in food consumption by two sexes, health, and nutritional status (Reitsema and Vercellotti 2012; Somerville et al. 2013). The stable carbon isotopic values may differentiate between the ingestion of C_3 and C_4 terrestrial resources (Salazar-Garcia et al. 2014).

The carbon isotopic ratios can be quantified from both the organic and inorganic components of bone (i.e. collagen and apatite) respectively. In bone collagen, the large portion of the carbon content (75%) is obtained from the intact amino acids of the proteins whereas about 25% portion of carbon content is obtained from carbohydrates and lipids (Fernandes et al. 2012; Pestle et al. 2015). As a result, $\delta^{13}\text{C}$ estimated from collagen is more indicative of the protein portion of diet. The high diagenetic susceptibility of bone and dentine hydroxyapatite likely may compromise their isotopic integrity (Jim et al. 2004). The $\delta^{13}\text{C}$ values estimated from apatite may be quietly unreliable and may not

reflect the biogenic carbon content. Bone regularly remodels during life, resulting in changes in isotopic composition associated with changes in diet, geographical place and illness. The turnover rate of bone remodelling changes according to gender, age, type of bone, elements present within the bone (Mbeki et al. 2017). In contrast to bone, dentine is the organic component of dental elements that usually don't remodel at all. As it is formed during the early years of life, the carbon isotopic ratios of human dentine can be used to trace childhood diet (Mbeki et al. 2017). The stable nitrogen isotopic ratio of bone and dentine rises by 3-5% with every trophic level and it is used to differentiate diets abundant in plant proteins from those abundant in animal proteins.

Oxygen isotope analysis of human skeletal remains is an effective method for the investigation of the place of origin (local or non-local), reconstruction of weaning events, migration routes (direction and range), mobility of an individual in different areas of the world and reconstruction of climatic changes over centuries (Huertas et al 1995; Wright and Schwarcz 1996, 1998; Stephan 2000; Laffoon et al. 2012; Eerkens et al. 2013). The oxygen isotopes are abundantly present in bone tissues, though its presence is largely determined by environment temperature and hydrological balance of the ecosystem at that time and influences by latitude, altitude and humidity of the area also (Luz et al 1984; Kohn et al 1996). The biochemical environment of an individual's childhood origin can be examined from isotope analysis of tooth enamel because enamel mineralizes during childhood and it does not undergo any substantial remodelling throughout life. Tooth enamel is much more resistant to diagenetic alterations of burial

environment than the bone, so tooth enamel isotope outcomes are considered more reliable (Laffoon et al. 2012). The isotopic compositions of environmental water and the foods consumed are directly related to oxygen isotopic contents of human skeletal tissues such as bones and teeth (Luz et al 1984, Podlesak et al. 2008). The positive correlations have been confirmed between oxygen isotope composition of environmental water and the phosphate groups of bone and tooth apatite (Luz and Kolodny 1989). Lisowska-Gaczorek et al. (2017) determined the origin of an early medieval group of individuals by comparing average monthly $\delta^{18}\text{O}$ levels and its variability in precipitation water and phosphate groups of the excavated bones and reported that variations in oxygen isotope composition of water precipitation from human bones excavated from 279 locations in Poland were enough to determine place of origin, reconstruction of migration routes and weaning events of individuals.

Strontium isotope analysis is useful for analysing migration extents and routes (direction and range) and studying residential mobility of ancient human populations. The comparison of ^{87}Sr (heavier)/ ^{86}Sr (lighter) values in human skeletal remains and the referenced environmental variability range can help track the geographic location inhabited by an individual or the group (Szostek et al. 2015). Strontium isotopes ($^{87}\text{Sr}/^{86}\text{Sr}$) obtained from dietary items can be related to geological environment of the place from where the food was procured. The strontium isotope values got embedded in the local soil, plants, animals and humans by the ingestion of water and foods. The local reference values from biological material ($^{87}\text{Sr}/^{86}\text{Sr}$ values) are important to recognize measurements

in human skeletal remains as strontium isotopic value is affected by different factors in biological processes (Naumann et al. 2014). The Isotope Ratio Mass Spectrometer (IRMS), Multi Collector Inductively Coupled Plasma Mass Spectrometry (MC-ICP-MS), and Elemental Analyzer (EA) are most commonly used by most of the researchers to analyse stable isotopic content of past bones and teeth. Bone collagen & apatite, teeth enamel or dentin samples have been used in stable isotope studies to reconstruct behaviours and subsistence practices of past people.

From the review of various studies we observed that human tooth is a better choice than bone for determining geographic origin, childhood migrations, infant breastfeeding and weaning practices, paleodiet and subsistence practices of past populations from its stable isotope analyses. The tooth isotopic signatures represent a particular period of time in each person's lifetime which, in turn, doesn't experience any substantial remodelling during childhood and adolescence. Bone remodelling is a continuous process which imprints the dietary, residential or illness changes during an individual's lifetime (reflecting environmental conditions, e.g. potable water), in the form of changes in isotopic composition associated with such changes. The turnover rate of bone changes according to biological sex, age, type of bone, element and location within the bone (Hedges et al. 2007; Debono et al. 2011). Waterlow (2006) stated that the bone collagen turnover changes within the bone. The immature bone collagen is degraded within hours, whereas the mature collagen indicates approximately no turnover, so average rate for all bone collagen is based on the ratio of the ma-

ture and immature collagen fractions related to individual's life stage and level of stress. However, dentine does not remodel as it get formed during early life of the individual, therefore, carbon isotope analysis of human dentine is considered as a useful tracer to reconstruct childhood diet (Nelson and Ash 2010). The isotopic composition of the nitrogen in collagen reflects that of dietary protein. Stable nitrogen-isotope analysis is an especially striking method to investigate the relationships between fertility, mortality, reproductive and weaning behaviour in past and present populations. Fertility and weaning behaviours depend upon many factors like the types of foods available, cooking practices, the quality of medical facilities available to children, and the society's perceived need for more labour force (Schurr 1997, 1998).

By analyzing human bones and teeth, we can reconstruct an individual's diet both during their childhood as well as within the last few years of the death (Lamb et al. 2014). The main aim of present review was to sum-up the available information on the following topics in a nut-shell:

Provenance of human skeletal remains

The use of stable isotopes, as 'chemical signatures of identity', is relatively a newer analytical tool for forensic investigations, though such analyses have been performed for over 30 years for anthropological or bio-archaeological purposes. The naturally-occurring differences in isotopic composition of evidentiary materials like hair, bones or teeth usually act as fingerprints of specific localities and thus, can be compared with materi-

als of known origin to provide vital clues for solving a case. Stable isotope analysis of human skeletal remains has proven useful for provenance of such remains in forensic contexts (Beard and Johnson 2000; Fraser et al 2006; Meier-Augenstein and Fraser 2008; Holobinko et al. 2011). The oxygen isotopic composition of human bones and teeth vary in different geographical regions due to variations in aridity and isotopic composition of drinking water used by the victims. The strontium and oxygen isotopic estimates of human teeth reflect the isotope values of food (soil, bedrock) and water, respectively, consumed by them during early life. From isotopic analysis of strontium and oxygen in tooth enamel of two unidentified human skeletons recovered from Netherlands, Font et al. (2015) reported that the victims belonged to World War II soldiers of United Kingdom who fought. The $\delta^{13}\text{C}$ and $\delta^{18}\text{O}$ isotope ratio of tooth enamel was applied to discriminate between the skeletal remains of American soldiers and Vietnamese victims (Regan et al. 2006). Stable carbon and nitrogen isotopes of bone show food ingestion sequences, which frequently differ in geographical areas due to cultural dietary differences. Bartelink et al. (2014) have reported the role of stable carbon and nitrogen isotope analysis of bones to discriminate between Cambodian, Vietnamese and US war victims in a mixed sample of human remains. Similarly, the $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ analysis of teeth confirmed the identity of US and Japan soldiers with higher accuracy and reliability thresholds (Somedá et al. 2016). Thus, stable isotope analysis has been confirmed as useful method of discriminating between human skeletal remains collected from different geographic areas of origin with high specificity. The use of

life tables from skeletal series has been widely and justifiably criticised (Woods 1992) so some alternate methods like stable isotope analyses or histomorphometric methods have been suggested to correlate the age-at-death estimates demographic processes in ancient individuals. Histomorphometry is a technique based on measurements and determination of number and density of basic bone structural units (osteons) and it is considered as a useful complementary method for age at death estimation (Mnich et al. 2017).

Breastfeeding and Weaning practices

Stable isotope analysis is an important method in human biology to understand the demographic characteristics like fertility, reproductive behaviour, mortality, breastfeeding and weaning practices of past populations. The complex relationship between weaning patterns (timing or rate) and fertility trends in past populations can be compared from the stable nitrogen isotope ratios of the proteins extracted from human skeletal remains (Schurr, 1997, 1998). The infant breastfeeding and weaning practices (BWP) of past populations can be reconstructed from stable carbon and nitrogen isotope analysis of ancient human skeletal remains (Howcroft et al. 2012; Beaumont et al. 2013; Henderson et al 2014; Tsutaya et al. 2014). Tooth enamel is a better source for reconstruction of breastfeeding and weaning practices of ancient and modern humans, though other bioarchaeological evidences of human origin have also been widely studied. The BWP is an important estimator of dietary, health and fertility status of

past individuals (Campbell and Wood 1998, Lewis 2007; WHO 2009, Humphrey 2014) The $\delta^{15}\text{N}$ isotopic values of infants, which were similar to their mothers before and immediately after their birth, become 2 to 3% higher after several days of breastfeeding as they start receiving their protein from milk (Romek et al. 2013). The variation in infant $\delta^{15}\text{N}$ isotopic values is due to the trophic level effects and variations in mother's diet (Hedges and Reynard 2007). By measuring $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ isotopic values of subadult tissues like bones and teeth of different ages; the level of breast milk ingestion at particular ages and also the components of complementary foods can be easily estimated. Chinique de Armas et al (2017) applied Bayesian probability models to carbon and nitrogen isotope ratios to assess the ages of start and end of weaning and probable food sources used for weaning for different age juveniles. Stable nitrogen isotopic ratios of prehistoric bone and teeth specimens can be used for comparing weaning and fertility behaviours of past populations, though reconstruction process is very complex; both biologically as well as statistically (Schurr 1998). The weaning period is also detectable by shifts in $\delta^{18}\text{O}$ levels when the infant stops breastfeeding and gets water supply from sources other than mother's breast-milk (Dupras and Tocheri 2007). Nitrogen isotope analyses of the human skeletal remains have shown that weaning started from the age of 6 months to 1 year, and it ended between the ages of 2 and 4 years for children (Kwok and Keenleyside 2015). Stable carbon and nitrogen isotope analysis suggested that children were usually weaned on to dairy foods and cereal diet only which, later on, shifted to dairy

foods, cereals, vegetables and fruits in the childhood (Schmidt et al. 2016).

Geographic Origins

Stable isotopes can also contribute significantly for determining the geographic origin of past human populations. The oxygen and strontium isotopes get embedded in biogenic phosphates of teeth during their formation and do not change throughout lifetime as tooth enamel never undergoes remodelling (Webb et al. 2013). Individual bones and teeth having same $\delta^{18}\text{O}$ isotopic values are supposed to have been born and died in the same geographic area whereas individuals showing different $\delta^{18}\text{O}$ isotopic values are presumed to have born in same area but died in another migrated area. Oxygen isotope ratios of human hard tissues, which get deposited from water locally available to the individual, can act as tracers of geographic origin. Most of the oxygen atoms in human skeletal materials come either from drinking water or the foods consumed as plant tissues meat, milk, eggs etc. The oxygen isotope analysis of human skeletal remains can be used to differentiate locals from non-locals if the oxygen isotope values of the geographic area from which immigrants originated differ from those of the area to which they have relocated (Keenleyside et al. 2011). Thus, the isotopic composition of bio-apatite is directly related to the isotopic composition of environmental water of the area where an individual lived during the phase of tissue formation. Lightfoot et al. (2016) observed that range of $\delta^{18}\text{O}$ isotopic values within most populations in bioarchaeological contexts is probably greater than 3‰ due to physiological factors, drinking water alterations, food

and drink importations, and the use of various sources of water having different $\delta^{18}\text{O}$ values. Isotope databases for different geological areas and different human tissues is possible for comparative purposes (Font et al. 2015). Thus, oxygen and strontium isotopes can map the isotopic outline of the area where an individual consumed the water and food during early childhood life (Bastos et al. 2016). In addition to the prevailing environmental and geological conditions, the physiological and metabolic factors of an individual also play important role in deposition of oxygen isotopes ($\delta^{18}\text{O}$) in the bone tissues, so the origin and migration history of an individual should be carefully interpreted from oxygen isotope ratios estimated from bones of the deceased (Lisowska-Gaczorek et al. 2016) The $\delta^{18}\text{O}$ ratios in bone tissues were found to increase after 40 years of age in females only, so sex and age of the bone should be estimated firstly before oxygen isotope estimates for interpretations of past human populations (Stepiczak et al. 2014).

Migration routes and mobility patterns

Strontium isotope analysis is widely used for examining ancient human migrations because strontium is strongly defined geographically. It's an important chemical signature of migration history of past human populations to opine whether they were local or non-local to the site (Oelze et al. 2012). This technique has been extensively used in anthropology ever since (Bastos et al. 2016) in studies from different parts of the world such as Oceania (Shaw et al. 2010), Europe (Knudson et al. 2012) and Asia (Gregoricka 2013;

Kenoyer et al. 2013), Strontium isotopic values of enamel reflect signatures of local plants and geology of a person where he lived. Due to slow bone turnover rate in adult humans, the strontium is incorporated into the bone throughout their lifetime period. In cortical and trabecular bone, turnover rates of about 3% and 26% of strontium per year have been estimated respectively (Parfitt 1983; Price et al. 2000), whereas the enamel does not show turnovers after its formation. In human tooth enamel and bone, strontium isotope ratios show the source of a diet around the time of birth and death of an individual, respectively. However, the variability in $^{87}\text{Sr}/^{86}\text{Sr}$ values across various geographical areas is relatively low, which frequently complicates reliable interpretation of migration history estimations. So, it becomes important to know the extent of diversity in $^{87}\text{Sr}/^{86}\text{Sr}$ isotopic levels among individuals moving to diverse localities and those restricted to specific geographic regions (Shaw et al. 2010). While comparing isotopic ratios of human skeletons with the range of biologically available strontium, it must be kept in mind that not all strontium from the region is absorbed by the body but only in the needed proportion as food (Knudson and Tung 2011).

Strontium isotopic studies examine populations in which the diet is supposed to be terrestrial and where non-local individuals can be identified by comparison with the isotopic composition of local sources for bio-available strontium, for example, plants and animals. Bentley et al. (2004) reported that strontium isotope analysis of human skeletal remains recovered from early farming sites in Central Europe showed a higher degree of residential movement among populations. Because agriculture is generally

related with increasing sedentism, it may reveal that few early farmers buried at these archaeological sites were the first inhabitants who genuinely migrated to their place of interment during their lifetime. There might have been migrations within a particular population, but they could remain unnoticed. It is important to determine to what extent the diversity of $^{87}\text{Sr}/^{86}\text{Sr}$ isotopic levels results from the individuals moving in relation to geologically varied surroundings, and to what extent it conforms to local variation between individuals.

Paleo-diets and Subsistence practices

The dietary and subsistence patterns of individuals of past populations can be also be reconstructed from isotopic analyses of their organic remains (Sealy 2001). The reconstruction of trends and patterns in diet and mobility has become the cornerstone of Bioarchaeology (Lewis et al. 2017). Pastoralism, hunting, fishing, cereal agriculture, group hunting/mobility or agro-pastoralism subsistence patterns/strategies have been identified from such analyses (Svyatko et al. 2013). Stable isotope analysis of human bones and teeth can recognize both intra and inter-population variation in diet. Various studies have revealed that the subsistence practices of the past human populations experienced extensive alteration (Wang et al. 2010; Hou et al. 2013). Stable isotope analyses have supplemented the archaeological or ethno-historic evidences of subsistence patterns or paleodietary status of past populations (Stantis et al. 2015). Stable carbon isotope analysis from skeletal remains has proved important in estab-

lishing the contribution of millet, a C₄ plant, to diet in the period after domestication started. The contribution of C₄ plants to the diet of past human populations can be estimated by stable isotope analysis (López-Costas et al. 2015). The ratios of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ isotopes in human bone collagen are increased by the regular consumption of millets, maize and aquatic resources while nitrogen isotope ratio should remain uninfluenced, permitting to differentiate C₄ plants from the consumption of aquatic resources which are mainly enriched in nitrogen isotope values (Reynard and Hedges 2008). Sealy (2010) conducted a study on 160 adult human skeletons recovered from forelands of southernmost Arica for stable isotope analysis and reported that ancient people ate mixture of marine and terrestrial foods, but terrestrial C₄ grasses were of quietly prominence. Sheep and probably cattle first came into existence in archaeological sites approximately around 7500 BP and there was no significant change in the isotope ratios of human skeletons in the first millennium AD whereas in the second millennium AD, people started to eat considerably more C₄ grasses and domestic stock, especially cattle became more essential in past population's diet. The isotopic values of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ of local fauna of any archaeological site can be used to clarify the importance of freshwater resources in the diet of the ancient human found near the site (Bocherens et al. 2016). Carbon isotopic values in tooth enamel give important way of linking people to their place of origin through dietary patterns. Carbon isotopic values in foods such as maize or marine resources, which pursue the C₄ photosynthetic pathway, are much more enriched than C₃ pathway foods, for example freshwater fish, terrestrial

game, (Wright 2006). Bone collagen is used more frequently to reconstruct diet during last years of life, but comparisons of carbon isotopic values in bone and teeth indicate that children consumed the related proportions of food in comparison to their adult counterparts. The stable carbon and nitrogen isotopic analyses can provide archaeo-zoological and paleo-botanical evidences of past mixed subsistence economies to help reconstruct their dietary and social structures (Reitsema and Kozłowski 2013).

Dietary calcium is primarily obtained from dairy products and vegetables; meat and water provide little calcium compared to milk and plant materials. Calcium isotopes of human bone apatite may serve as useful archaeological source to reflect past human diet (mainly dairy or plant products), with an average fractionation of 0.65–0.75‰ relative to the diet (Chu et al. 2006; Reynard et al. 2010, 2011, 2013). Reynard et al. (2011) concluded that in case of mixed dietary habits, milk consumption cannot be segregated out as the important source of calcium isotope ratios estimated from the bones.

Difficulties working with stable isotope analyses for anthropological purposes

The bone apatite and tooth dentine are more vulnerable to archaeological diagenetic alterations like contaminations, microbial destructions, degradations and substitutions than the tooth enamel (Hedges 2002, Hinz and Kohn 2010). The consideration and evaluation of such alterations becomes important for accurate reconstruction of biological signals from isotopic concentrations in archaeological

materials. So, isotopic analyses should be done with extracted collagen and tooth enamel apatite than bone apatite or dentine. The isotopic and elemental signatures in enamel apatite can remain preserved for millions of years after tooth fossilization; however, collagen portion generally retains chemical structures for few thousand years only, unless buried in special conditions like as permafrost (Hinz and Kohn 2010). Oxygen isotope analysis is preferred to nitrogen estimating the weaning ages of earlier humans (Tsutaya and Yoneda 2013). From isotope analyses, it may not be possible to discriminate two populations who were culturally different but lived in similar climatic conditions or ate similar kinds of foods. Contamination issues may challenge the utility of stable isotope analyses for anthropological purposes as there are chances that the isotopic signature picked up in a tissue might have come from another source than the human body itself; either via leaching in with substrate water or from exposure to local soils or even at the laboratory during washing treatments etc. Stable isotopic studies may sometimes give inaccurate or invalid results due to overlapping values of carbon and nitrogen isotopes in different dietary items. The diagenetic alterations may affect variety of isotopes and tissues in different ways, so analyst must be very much aware of the changes that may affect the resulting isotopic values. Sometimes, the isotopic compositions of terrestrial and aquatic foods overlap to disguise the relative contribution of two types of human diets in the past (Reitsema and Kozłowski, 2013). Stable nitrogen-isotope ratios have not been widely used to study prehistoric breastfeeding and weaning practices as this technique is relatively new and is not widely known

and also preparation of samples for nitrogen isotope analysis is very difficult, time-consuming and prone to contamination from atmospheric nitrogen. Relatively large samples of juvenile skeletons spanning all or most of the ages prior to adulthood are required for concluding some valid generalisation to determine ancient weaning behaviour from nitrogen isotopic ratios (Schurr 1998)

Conclusions

Isotope analyses play a very important role in the verification of hypotheses concerning diet, the weaning process and mobility of pre-historic human populations. Stable isotope analysis may be an alternate method of identification when DNA techniques fail to yield the desirable results or the knowledge of recent geographic life-history of the deceased may prove vital in a forensic case solution. We have reported the current status of stable isotope analysis to determine breast feeding and weaning practices, geographic origin, migration history, paleodiets and subsistence practices of past people from human skeletal remains of the victims. Analysis of stable carbon and nitrogen isotope showed that children were weaned onto a diet mainly of dairy foods and cereals, after that the childhood diet was consist of dairy products, cereals, vegetable and fruits. $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ isotopic values of sub-adult's bones and teeth of different ages may be useful in the reconstruction of the constituents of complementary foods and the degree of breast milk consumption. Humans whose bones and teeth reveal alike $\delta^{18}\text{O}$ isotopic values are supposed to have been born and died in the same place, while those who indicating different values are supposed to have born in

same geographic region but died in another migrated region. The carbon and nitrogen isotopic values of local fauna of any archaeological site can be used to find the importance of freshwater resources in the diet of the ancient human found near the site. Stable isotope analyses usually provide clues about the contribution of C_4 plants like millets to the diet or prehistorians. We conclude that stable isotope analysis is a best method in provenancing human skeletal remains. But, future research is required to explore the role of stable isotope analysis of other elements in provenancing and determining breast feeding and weaning practices, geographic origin, migration history, and paleodiet and subsistence practices from human skeletal remains. Isotopic analyses have been increasingly used not only to trace material goods or food products of ancient people but also studying some inter-group relationships, establishing trade or migration routes and in medicolegal forensics (English et al 2001, Liu et al 2014, Holobinko 2011, Szostek et al. 2015)

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Authors' contributions

JSS conceptualized and designed the framework of this review article by contributing substantial scientific inputs, writing and editing the contents according to journal style, scrutinising the re-

lated articles and co-ordinating the opinions of the other author. JK contributed in scrutinising, selecting and comparing scientific articles/literature, and preliminary analysis of the contents in context to the aims and objectives of this manuscript.

Conflict of interest

The authors declare that there is no conflict of interests regarding the publication of this paper.

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