A147 Mitochondrial DNA and Stable Isotope Analyses as Molecular and Chemical Signatures of Identity of Victims: A Combined Approach for Provenancing Unknown Skeletal Remains From India

Jagmahender Singh Sehrawat, PhD, Panjab University, Dept of Anthropology, Sector 14, PU Main Campus, Chandigarh 160014, INDIA; and Raj Kamal, PhD, Panjab University, Dept of Anthropology, Sector 14, PU Main Campus, Chandigarh 160014, INDIA*

The goal of this presentation is to scrutinize the combined forensic suitability of molecular and chemical methods of identification for human skeletal remains recovered from an abandoned well situated beneath a religious structure in a North Indian suburb of Ajnala in an attempt to satisfy the major objective of establishing their identity and origin status (local or non-local to the site) from the mitochondrial DNA (mtDNA) and stable isotope analyses of the recovered teeth and bones.

This presentation will impact the forensic science community by illustrating how the combined approach of application of mtDNA and stable isotope analyses has unraveled the scientific truth about these non-scientifically excavated human skeletal remains from a well by amateur archaeologists. The forensic anthropological findings have been corroborated by the molecular and chemical analyses of the remains, which will help in arriving at a number of valid conclusions regarding the forensic identity of these remains.

Unknown human remains have been reported from a number of disaster or multiple burial sites of forensic or bioarchaeological nature, whose identity establishment is of prime concern for a forensic anthropologist. The bones and teeth serve as anthropological, molecular, and chemical signatures of identity of the deceased as their physical structures, molecular signatures, and chemical compositions retain a number of specific individual imprints. Teeth are considered storehouses of invaluable biological, physical, and chemical information, and are crucially required for various forensic anthropological purposes, such as provenance or the establishment of biological identity (age, sex, ethnicity, occupation, migration pattern), exposure to pollutants or taphonomic/traumatic insults, ancient DNA analyses, estimation of dietary practices and subsistence patterns, paleopathology, etc. The small size and mineralized status of human teeth imparts them with a resistance against all types of decompositions and degradations, thus making them suitable for answering a wide range of forensic questions. In addition to certain gender-, race-, and age-dependent variations in human teeth, certain culture- and occupation-specific features also play an important role in the identification process. The disruptive and challenging life events (illness, malnutrition, starvation), especially during childhood, are impregnated in tooth enamel to reveal such severe and stressful life events.

The small size and high copy number of mtDNA ensures a higher probability of intact DNA isolation from severely degraded human remains found in forensic situations, which can be further amplified and studied using DNA markers from nuclear DNA as well. The human molars (mandibular), petrous bone, and femur are comparatively better and thus preferred sources for mtDNA extractions, compared to other traditionally used DNA sources.1 The stable isotope analyses of unknown human skeletal remains have provided novel approaches for their provenancing in forensic or bioarchaeological contexts.2 The isotopic ratios of bones and teeth have proven to be fingerprints of locality, migration patterns, subsistence activities, and dietary habits, such as milk or meat consumption by the victims, infant breast-feeding practices and weaning patterns, prehistoric diets, etc.3-4 Stable isotopes of bone and teeth vary significantly among different geographical regions due to diverse cultural dietary patterns and environmental factors (such as aridity, elevation, and distance from large bodies of water). Thousands of bones and teeth were recovered from an unused well situated beneath a religious structure at Ajnala in April 2014, after being identified in a book.5 These remains were provided to this study in 2015 to establish their biological identity.

Two hundred-fifty-six mandibular molars were randomly selected for mtDNA extraction, quantification, and amplification, and whole genome sequencing was performed for few samples. An equal number of teeth and some bones (femur, humerus, vertebrae, clavicle, metatarsals, and metacarpals) were processed chemically for stable isotope analysis of carbon, hydrogen, oxygen, nitrogen, and strontium. The tooth sample was completely settled in silicone rubber, a dermal cut-off wheel and bids were used to cut the root and make a hole through it, respectively. Approximately 50mg of powder was taken and processed for DNA extraction and analysis at 108 variable positions to genotype the haplogroup-defining motifs of the entire mtDNA. The ancient DNA sequences and haplogroups were compared with individuals of geographical areas to which the victims supposedly belonged. Samples were prepared for stable isotope analysis according to standardized techniques and the isotopic compositions were estimated from different dental and skeletal remains using Isotope Ratio Mass Spectrometer (IRMS), and were then compared with the thresholds available for comparisons for different periods and regions.2

Keeping in mind the enormous diversity of the Indian population, the ethnicity and geographic area of specific mtDNA diagnostic markers (N=104) were used to assign the haplogroups, and it was observed that out of 49 samples, the haplogroup pattern of 35 samples were similar to North Indian populations, whereas 14 samples exhibited more similarity with present-day Pakistani and Iranian populations. The frequency distribution pattern of all haplogroups across Indian, Central Asian, and Middle East populations assigned the affinity of the Ajnala skeletal remains to the Punjab and Uttar Pradesh states of India. These findings were contrary to the written versions and anthropological observations regarding these remains.5,6 The stable isotopic interpretations and the whole genome studies further corroborated these observations, thus unravelling the scientific truth concerning these remains.

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*Presenting Author