ISOTOPIC TRACING OF MORTALITY CAUSED DUE TO INFECTIOUS DISEASE

ინფექციური დაავადებით გამოწვეული სიკვდილიანობის იზოტოპური გამოკვლევა

ACHARYA BALKRISHNA
PhD, Vice-Chancellor
Division of History & Archaeological Research, Patanjali Research Foundation (Trust), Uttarakhand, India.
Department of History, University of Patanjali, Uttarakhand, India
e-mail: historymeeting2023@patanjali.res.in
ORCID:0009-0009-1978-9885

SNEHA UNNIKRISHNAN
M.Phil, Assistant Scientist
Division of History & Archaeological Research, Patanjali Research Foundation (Trust), Uttarakhand, India
e-mail: sneha.unnikrishnan@patanjali.res.in
ORCID: 0009-0001-8468-3624

PANUSHA NAGARAJAN
M.A, Assistant Scientist
Division of History & Archaeological Research, Patanjali Research Foundation (Trust), Uttarakhand, India
e-mail: panusha@patanjali.res.in
ORCID:0009-0002-6730-1169

RASHMI MITTAL
PhD, Head of the Department
Division of History & Archaeological Research, Patanjali Research Foundation (Trust), Uttarakhand, India
*e-mail: rashmi.mittal@patanjali.res.in
ORCID: 0009-0000-7649-1510

Abstract

Epidemics have affected human history since its emergence. The transition to sedentary agriculture, human migration, and animal domestication led to a rise in the occurrence of infectious diseases. As mortality rates continued to increase within various societies, there developed a crucial need to examine the root causes contributing to the development and progression of these diseases. Isotope analysis using strontium ($^{87}Sr/^{86}Sr$), oxygen ($\delta^{18}O$), carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) were conducted on samples from archaeological sites in Winchester, Hull, Chile, and Saint-Doulchard to investigate the potential causes and contributing factors of these diseases. Studies have revealed that these sites were infected by lepromatous, treponematosis, tuberculosis, and the Justinian plague. The data further revealed information regarding the provenance, ecological setting, and mobility patterns of population afflicted by these diseases. This review article highlights isotopic studies as a significant marker for depicting the mortality associated with infectious diseases in ancient societies.

Keywords: $\delta^{13}C$, $\delta^{15}N$, $\delta^{18}O$, Justinian Plague, Lepromatous, Treponematosis, Tuberculosis

აბსტრაქტი

ეპიდემიებმა გავლენა მოახდინა კაცობრიობის ისტორიაზე მისი გაჩენის დღიდან. უმოძრაო სოფლის მეურნეობაზე გადასვლამ, ადამიანთა მიგრაციამ და ცხოველთა მოშინაურებამ გამოიწვია ინფექციური დაავადებების შემთხვევების ზრდა. რადგან სიკვდილიანობის მაჩვენებლები სხვადასხვა საზოგადოებაში კვლავ იზრდებოდა, გაჩნდა

გადამწყვეტი აუცილებლობა, შესწავლილიყო ამ დაავადებების განვითარებისა და პროგრესირების გამომწვევი ძირითადი მიზეზები. იზოტოპური ანალიზი სტრონციუმის (87Sr/86Sr), ჟანგბადის (δ18O), ნახშირბადის (δ13C) და აზოტის (δ15N) გამოყენებით ჩატარდა ვინჩესტერის, ჰალის, ჩილეს და სენ-დულშარის არქეოლოგიური ძეგლებიდან აღებულ ნიმუშებზე, რათა გამოკვლეულიყო ამ დაავადებების პოტენციური მიზეზები და ხელშემწყობი ფაქტორები. კვლევებმა აჩვენა, რომ ეს ადგილები დაინფიცირდა ლეპრომატოზით, ტრეპონემატოზით, ტუბერკულიოზით და იუსტინიანეს ჭირით. მონაცემებმა ასევე გამოავლინა ინფორმაცია ამ დაავადებებით დაავადებული მოსახლეობის წარმოშობის, ეკოლოგიური გარემოს და მობილობის ნიმუშების შესახებ. ეს მიმოხილვითი სტატია ხაზს უსვამს იზოტოპურ კვლევებს, როგორც მნიშვნელოვან მარკერს უძველეს საზოგადოებებში ინფექციურ დაავადებებთან დაკავშირებული სიკვდილიანობის ასახვისთვის.

საკვანძო სიტყვები: $\delta^{13}C$, $\delta^{15}N$, $\delta^{18}O$, იუსტინიანეს ჭირი, ლეპრომატოზი, ტრეპონემატოზი, ტუბერკულიოზი.

Introduction

From ancient times to the present, human population across the world has experienced many infectious diseases, which have affected the development of society. Infectious disease outbreaks have occurred throughout the globe, caused by exposure to a remarkable diversity of pathogens, parasites, and viruses thereby challenging human health (Larsen & Crespo, 2022). Earliest records from ancient Greece and Egypt indicate mortality from epidemics like diphtheria, leprosy, smallpox, tuberculosis (TB) etc. Evidences of smallpox were also found in Chinese texts dating around 581-907 BCE (Van Der Kuyl, 2022). The mortality from infectious diseases had a significant impact on culture, politics, and socio-economic aspects. Even in present scenario, a significant ratio of population is affected by infectious diseases where the cure is not readily available. So it is important to study the past occurrence of these diseases to better grasp its pathogenesis and clinical aspects that will help to combat the diseases in present times as well (Larsen, 2018; Sakai & Morimoto, 2022).

Archaeological and literary evidence from different regions offers valuable insights into the history of infectious diseases amongst ancient human populations. In Asia, the history of infectious diseases in human populations can be traced from classical medical and political writings. The descriptions of infectious diseases such as leprosy can be found in the *Sushruta Samhita* and Kautilya's *Arthashastra* from the 6th Century BCE. Leprosy records from human skeletal remains were obtained from Balathal in Rajasthan, India from 2000 BCE (Robbins et al., 2009). In Africa, analysis of mummies from Abydos, Upper Egypt has shown evidences of tuberculosis dating to 3500–2650 BCE (Zink et al., 2003). Similarly, in Europe cases of Justinian plague (541-543 CE) caused by the bacterium *Yersinia pestis* was reported. Archaeological evidence depicting Justinian plague was found at Aschheim cemetery in Bavaria, Germany, from human remains dating to 6th Century CE. This plague is known to have resulted in mortality of millions of people which led to the decline of Byzantine Empire and marking the transition from the Classical to the Medieval period (North, 2013; Wagner et al., 2014). Archaeological evidence of tuberculosis dated between 17,000-20,000 years ago were obtained from Natural Trap Cave in Wyoming, USA (Hershkovitz et al., 2015). In South America, evidence of *Trypanosoma cruzi* infection (Chagas disease) was found in a female mummy from Southern Peru dated to 1451-1642 CE (Panzer et al., 2014). These evidences clearly indicate that epidemics were spread throughout the world in ancient times.

Techniques like isotope analysis, ancient DNA analysis, paleoprotemics, lipid analysis has led to advancements in the study of ancient infectious diseases. This has led to better understanding of past heath concerns thus guiding medical research of the present (Anastasiou & Mitchell, 2013; Tomei et al., 2023). Isotope analysis plays an important role in understanding the infectious diseases of the past. Isotopes are variations or forms of a chemical element with varying neutron counts but the same number of protons and electrons. It helps researchers trace from where a certain disease originated and how it has spread by comparing the chemical composition of hair, bones, and other tissues to the chemical composition of water and soil in certain geographical regions. Apart from playing a significant role in medical research, use of isotopes have also been explored to study the mortality caused due to infectious disease. Besides studying the spread of certain diseases, isotope analysis can also help in understanding host body and immunity level. Isotopes namely carbon ($\delta^{13}C$), nitrogen ($\delta^{15}N$), strontium ($^{87}Sr/^{86}Sr$), and oxygen ($\delta^{18}O$) are commonly used to study mortality pattern caused due to the diseases. These analyses reveal disease dynamics, understanding of illness-mediating variables, and the respective ecosystems (Reitsema & Holder, 2018; Sakai & Morimoto, 2022; Stantis & Kendall, 2022).

1. Historical Outline of Infectious Diseases and Mortality

The history of infectious disease and its associated mortality is inextricably linked to anthropogenic transitions dating back to the prehistoric era. Infectious disease including leprosy, tuberculosis and trepanematoses are known to cause significant mortality in the past. The prominence is attributed to their geographical spread, well-known disease progression and lengthy documentation period. These diseases documented in the paleopathological record are thus correlated with mortality in ancient populations (Henneberg et al., 2021). During ancient times infectious diseases has caused significant increase in mortality rate. These diseases are namely tuberculosis, leprosy, treponematoses.

Tuberculosis (TB) is amongst the deadliest infectious diseases. *Mycobacterium tuberculosis* bacterium is known for causing this infectious disease. Hippocrates (460 BC) in his book entitled “Book I, Of the Epidemics” has documented mortality caused due to this disease within few populations of Alexandria. In 1700, the disease was referred to as the “white plague”. During 18th Century tuberculosis was also labelled as “the robber of youth” attributing for mortality of young people (Mousavi-Sagharchi et al., 2025). Leprosy, also known as Hansen's disease, is one of the oldest illnesses caused by *Mycobacterium leprae*. This disease leads to deformity causing physical disability affecting millions of individuals worldwide. The social stigma has lead it to be known as “the death before death”. The earliest mentions can be found in ancient India around 600 BC. While during the Middle Ages (1000–1400 AD) in Europe, a period of widespread growth followed by a subsequent decline has been observed. By 1980s around 10-12 million were found to affected by this disease worldwide (Bennett et al., 2008; Santacroce et al., 2021).

Treponematoses is a collective term intended for pinta, yaws, bejel and syphilis caused by *Treponema* bacteria. The spread of this disease is highly debatable with four theories namely Columbian, Pre-Columbian, Unitarian, and Evolutionary. Studies has showed that around 2½ million people may be infected with treponematoses worldwide (Collins & Powell, 2011). Apart from leprosy, treponematoses, tuberculosis, plagues also caused significant mortality among populations. Three major world pandemic occurred due to *Yersinia pestis* that drastically impacted world demography: The Justinian Plague (6th-7th Century), Black Death (14th Century), and Bubonic Plague/ The Third Plague (1855-1959) (Sakai & Morimoto, 2022).

One of the first outbreaks, the ‘Justinian plague’ originated in Pelusium and transmitted through the Mediterranean area, its outlying areas and reached the borders of Ireland and Persia by 543 AD. Its high virulence lead to high mortality rate and the subsequent strain directly resulted in the decline of the Byzantine Empire (North, 2013). The plague resulted in a long-term series of biological disasters. Historians have identified 18 distinct waves of plague that affected the Near East and Europe up to the middle of 8th Century (Cohn, 2008). Second, major plaques is known as ‘Black Death’. This was a catastrophic multiregional event affecting between one-third to two-thirds of the European population, further impacting Asia and the Middle East. This later led to major outbreaks in different regions of Europe including Milan, London, Vienna, and Marseille between 17th-18th Century (Benedictow, 2008). The mortality rates reached up to 100,000 and 48,000 in Moscow and Messina respectively. Aftermath of this plague was believed to have affected until 1879 in regions like Egypt, Russia. Balkan (Cohn, 2008). The Third Plague or Bubonic Plague is believed to have spread from Yunnan, China via steamships into Hong Kong. The greatest death toll of this pandemic occurred in China, India, or subtropical regions but temperate areas remained unaffected. Contrarily to Justinian Plague or Black Death that were associated with millions of deaths, this plague primarily affected of port cities and in most geographic areas had fewer than 100 people die from the disease (Cohn, 2008).

The study of these infectious diseases and plagues demonstrates how significantly it contributed to mortality and caused continued changes in the populations over time. In this paper we will highlight the role that isotopes have played in understanding both past infectious diseases and the history of the diseased population.

1.1. Mortality Caused Due to Leprosy

Leprosy is a debilitating disease caused due to infection by *Mycobacterium leprae*. This disease is known to be “interwoven with civilization itself” and continues to be prevalent in the modern society as well. An understanding of the origin and transmission routes can provide valuable information about the history of the infectious diseases and the strategies used to treat them. Evidences of leprosy were found at Medieval European regions namely from United Kingdom, Denmark, Italy, Czech Republic, and Hungary (Robbins et al., 2009). The most severe form of leprosy is known as lepromatous or multibacillary leprosy. Classifications of leprosy is dependent on symptomatic features which is essentially dictated by the host's immune response (Froes et al., 2022). Archaeological investigations at the St Mary Magdalen leprosarium in Winchester, UK have provided evidences of disease prevalence with skeletal abnormalities (Roffey et al., 2017).

Specific burials at St Mary Magdalen leprosarium such as the Sk27 were analysed using radiocarbon dating, dietary isotopes, and ancient DNA analysis to correlate osteological observations with *Mycobacterium leprae* phylogeny and the geographic origins of the infected individuals (Roffey et al., 2017). The site's genomic characterization reveals that, the majority of isolates are of genotype 3I. Along with this, genomic characterisation also revealed polymorphisms and deletions compatible with strains ancestral to the 3I-1 and 3I-2 lineages prevalent in the southern United States and Mexico. Specifically, the pilgrim burial Sk27 was observed to be contaminated with a type 2F infection (Taylor et al., 2013).

Although modern type 2 strains are typically associated with Central Asia and the Middle East, they have been sometimes found in European archaeological sites, particularly in Scandinavia (Economou et al., 2013). The discovery of this strain in a pilgrim burial remains to be a matter of discussion because it supports the hypothesis that the growth of leprosy in Western Europe in the 11th and 12th Century coincided with an increase in the popularity of long-distance pilgrimage. Isotopic and morphological findings indicate that Sk27 was a non-local person (male) with potentially high social standing. Strontium and oxygen isotope analyses confirmed that the individual did not originate from the southern British chalk areas, and his diet was high in animal protein. To study the disease pattern, although his exact origin is unknown but his distinctive skull morphology shows physical connections with Southern Europe or North Africa, which is consistent with the existence of the type 2F strain and a history of frequent travel associated with this area. It is doubtful to state that whether he was a local resident before to his excursion or a return traveller. The multidisciplinary data demonstrates the complex interaction between medieval mobility and *M. leprae* transmission (Mendum et al., 2014; Roffey et al., 2017). The present study highlighted the use of strontium and oxygen isotopes to study the mortality caused due to leprosy at St Mary Magdalen leprosarium.

1.2. Mortality Caused Due to Treponematosis

Pinta, yaws, endemic syphilis, and venereal syphilis are the four clinical manifestations of treponematosis, a persistent bacterial infection. For instance, a study of twelve skeletal specimens were undertaken at a late Medieval cemetery in Hull, England. Here both strontium ($^{87}Sr/^{86}Sr$) and oxygen ($\delta^{18}O$) isotope analysis were employed for the study. This sample was divided evenly into two groups: a diseased group (n=6) with osteological evidence of treponemal infection, and a control group (n=6) without such pathological changes. The study included 674 dry bone specimens from documented cases housed in 22 European medical museums (Hackett, 1975; Roberts et al., 2013).

Naso-palatine destruction as well as considerable long bone changes caused by concurrent bone production and destruction processes were observed in specimens. Strontium and oxygen isotope analysis revealed that four of the twelve people likely originated outside of England, emphasising the role of human mobility in the epidemiology of treponemal disease during the medieval period (Hackett, 1975; Ortner, 2003).

Only two migrants revealed bone abnormalities consistent with probable and potential treponematosis. Additionally, evidences of advanced healed cranial involvement due to caries sicca were also found. This shows that the port of Hull's prominence acted as a trade and migration hub that would have aggravated the spread of the virus. However, it is unknown whether these individuals were infected before or after arriving in the city. Four other affected individuals, on the other hand, were identified as having been born in Hull and most likely catching the disease while being there. In order to comprehend the subtleties of how infections affected societies in the past, this work has demonstrated the possibility of connecting data on infectious diseases to isotope data for mobility. It emphasizes that, as is evident in clinical settings now, people were mobile during those times and had the capacity to disseminate their illnesses. It also shows that isotope analyses have a lot of potential for investigating how human movement affects the spread of disease and the consequent mortality (Collins & Powell, 2011; Roberts et al., 2013).

1.3 Mortality Caused Due to Tuberculosis

Bioarchaeological evidence indicated the presence of tuberculosis as a long-standing human disease. Evidence from the 'Old World' implies that the prevalence of tuberculosis grew with the switch to sedentary, increased agrarian lifestyles, which supported higher population densities and prolonged transmission of diseases. In the Americas, some of the earliest reported examples of tuberculosis come from northern Chile, notably mummified remains from the Tarapacá valley dated to around 700 AD (Arriaza et al., 1995; Aufderheide & Rodríguez Martín, 2011; Hershkovitz et al., 2015).

Mycobacterium tuberculosis is considered to be one of the causes of the epidemic in modern populations across the world. This pathogen can spread to extrapulmonary locations in humans, such as the skeletal system, even though it primarily targets pulmonary systems. The pathogen often stays in a latent or subclinical state during the initial stage of infection. It is thought that severe tuberculosis infections cause the host to undergo major metabolic changes, including catabolism, a negative nitrogen balance, and increased gluconeogenesis (Barry et al., 2009; Macallan, 1999).

Isotope analysis of host tissues depicted the physiological changes in carbon and nitrogen metabolism, as evident from increased $\delta^{15}N$ values and decreased $\delta^{13}C$ values through analysis carried out at Pica 8 site in northern Chile (1050-1450 AD). The use of single amino acid carbon isotope analysis to detect these metabolic fingerprints were carried out at this site. Isotope analyses can indicate disease-induced metabolic distress by analysing collagenous tissues, such as tendons and ribs, from people with TB-consistent skeletal lesions. Although the catabolic breakdown of muscle protein during infection is generally indicated by higher $\delta^{15}N$ levels (Katzenberg & Lovell, 1999).

Despite the expectation that bulk $\delta^{15}N$ would reveal catabolic states, there was no significant difference in nitrogen values between the pathological and control groups. Individuals with skeletal lesions had considerably lower (more negative) serine values inside rib collagen compared to those without lesions. Taking into account the diet, the research proved that changes in $\delta^{13}C$ serine were the result of changes in metabolic processes and not the effects of nutrition. Serine, a non-essential amino acid, is generated in the body by isotopic fractionation. As a result, the $\delta^{13}C$ values of serine and possibly other non-essential amino acids might be used as a stand-in to determine the distinct isotopic fractionation brought on by abnormal metabolic changes. This implies that compared to bulk isotope readings, single amino acid analysis offers a more sensitive diagnostic method for identifying chronic infectious illnesses like tuberculosis in ancient populations (Mora et al., 2021). Here carbon and nitrogen isotope were used to study infected population thus giving a better understanding of the mortality of their past.

1.4. Mortality Caused Due to Justinian Plague

During 6th Century AD, the reign of the Eastern Roman Emperor Justinian was marked by the Justinian plague caused due to *Yersinia pestis*. Believed to have originated in Egypt around 540 AD, affecting Constantinople by 541 AD and it quickly spread across Europe. This plague impacted both cities and isolated rural regions (Wagner et al., 2014).

Isotope studies were conducted at Saint-Doulchard, France, with a focus on corpses buried in a cemetery ditch to study death caused due to Justinian Plague. According to carbon and nitrogen isotope analysis ($\delta^{13}C$ and $\delta^{15}N$) of bone collagen, population of this region had a different diet than those in regular inhumations, with elevated $\delta^{15}N$ levels indicating a much higher intake of animal protein. The observed increase in $\delta^{15}N$ levels during the plague cannot be attributed to the infection's physiological impact. Additionally, although the catabolic breakdown of human tissues during acute famine can increase $\delta^{15}N$, this change is usually accompanied by a drop in $\delta^{13}C$ a tendency that is noticeably lacking in the Saint-Doulchard ditch burials in France (Vytlačil et al., 2024).

The exceptionally high concentration of *Yersinia pestis* DNA found in the ditch burials of Saint-Doulchard strongly suggests that the majority of those buried were direct victims of a plague pandemic rather than individuals who died during subsequent famine episodes or attrition mortality. The combination of molecular pathogen detection and burial context gives solid evidence for epidemic mortality rather than just dietary crises (Bos et al., 2011; Fornaciari, 2017). Interestingly, one young adult female harboured a novel strain of *Y. pestis* that was different from other samples at the site and showed the highest isotopic values in the dataset. These outlying genetic and isotopic markers point to a non-local origin for this person, suggesting that she may have acquired the particular bacterial strain before coming to Saint-Doulchard. Conclusively, the evidence suggests that rather than being victims of secondary nutritional stress, the ditch burials reflect a subgroup with a unique dietary profile, marked by higher animal protein consumption and victims of *Y. pestis* (Vytlačil et al., 2024).

It can be said that the evidences left behind by leprosy, treponematosis, tuberculosis, and the Justinian plague underscore their roles as important determinants of mortality and population dynamics throughout history.

2. Clinical Utility of Isotope Analysis in Studying Infectious Diseases

The scientific foundation for the development of isotope techniques in clinical research and diagnostics was laid by the ground-breaking studies of Schoenheimer and Rittenberg in the 1930s. Isotopes are perfect tools for the long-term evaluation in medical research because they are non-radioactive tracers. They provide a secure and morally sound way to validate new therapeutic interventions, such as gene therapies and novel pharmacological agents (Bodamer, 2001).

This analysis has emerged as a crucial tool in the bioarchaeological study of ancient infectious diseases, offering indirect yet compelling evidence regarding the ecological, dietary, mobility and mortality factors that affected pathogen transmission in historical populations. In paleopathology, isotopic signatures preserved in human tissues, including bone collagen, dentine, and enamel, can provide insights into diet, migration patterns, environmental exposure, and physiological stress, all of which are pivotal in the emergence and dissemination of infectious diseases (Reitsema & Holder, 2018; Stantis & Kendall, 2022).

The most common isotopes analysed in archaeological human remains include carbon ($\delta^{13}C$), nitrogen ($\delta^{15}N$), oxygen ($\delta^{18}O$), and strontium ($^{87}Sr/^{86}Sr$), which all reveal different aspects of the lives of our ancestors including composition of the diet and the position in food chain. These isotopes thus allow us to reconstruct the diet and nutritional status of our ancestors, which may affect the immune system and the prevalence of infectious diseases, if caused due to dietary changes.

Carbon ($\delta^{13}C$), nitrogen ($\delta^{15}N$), oxygen ($\delta^{18}O$), and strontium ($^{87}Sr/^{86}Sr$) are the isotopes used in order to gain an understanding of the way our ancestors lived, including their diet and their place in the food chain. In this way, the diet of our ancestors and its consequences for their immune systems can also be learned. High concentrations of certain isotopes, including $\delta^{15}N$ indicate biological evidence of stress on the body's physiology. Such findings suggest illnesses and nutritional deficiencies that make an individual more susceptible to disease due to their negative impact on the immune system. Overall, such isotopic research provides information about nutritional shortages that could be related to the transmission of diseases (Reitsema & Holder, 2018).

References of various isotopes in bone sample can vary according to diseases. For instance, reduced $\delta^{13}C$ can found with syphilis. Abnormal bone $\delta^{15}N$ and $\delta^{13}C$ levels can be observed in case of coeliac disease patients and victims of the Irish Famine, while depressed $\delta^{18}O$ values are detected with osteopenia (Stantis & Kendall, 2022). In addition to the reconstruction of the diet, strontium and oxygen isotope analyses are informative for the study of the geographic mobility and migration of humans. These are key aspects responsible for the transmission of infectious diseases. Along with other isotopes, strontium isotopes are significant because it represents local geology, allowing researchers to pinpoint the native location if they differ from the burial site. This method has also been employed to study the association between migration and the transmission of infectious diseases, to depict if any mobility pattern if involved (Mora et al., 2021; Vlok & Buckley, 2021).

Isotope studies also have a part to play in paleoepidemiological studies by establishing a link between environmental factors and subsistence strategies, and disease prevalence. For example, changes in diet that are a result of the shift to agriculture have been identified through isotope studies and are often associated with population density, nutritional stress, and epidemics of infectious diseases. By combining isotope studies with paleopathology and ancient pathogen studies, it is possible to elucidate complex relationships between human behaviour, environmental changes, and the evolution of infectious diseases (Larsen, 2018; Lewis et al., 2023). Although isotopic data cannot provide critical contextual data that helps to explain why particular individuals or groups were more susceptible to infection rather it is a critical component of a multidisciplinary approach to bioarchaeological research, which combines with other approaches such as skeletal pathology and molecular studies to elucidate the context of infectious diseases in past populations. Moreover, the bioarchaeological record presents a distinctive perspective on past infectious disease epidemiology. By analysing human remains like bones and teeth, it is possible to recreate past individual clinical experiences of our ancestors and their respective ecological condition (Larsen & Crespo, 2022; Mora et al., 2021).

3. Ethical Issues Associated with Isotopic Studies

The application of isotope analysis increasingly requires the consideration of intricate ethical frameworks. Since the field of archaeology essentially depends on the destruction of specimens in order to provide knowledge, ethical behaviour has become a major concern for practitioners. Due to the destructive nature of these analyses, which require a small portion of the sample, proper management of these analyses is a necessity. The preservation of the remaining sample is important for the integrity of the collection for future research possibilities (Squires et al., 2019). As a result, methodological decisions must be rigorously and continuously assessed at every stage of a project, from preliminary design and sampling to post-excavation evaluation. When using destructive techniques like mass spectrometry, such inspection is very necessary. Because archaeological material is a limited resource, disruptive sampling severely limits the possibility of future scientific study. Researchers are urged to consider how multiple analytical methodologies overlap, both practically and interpretively. Strategic integration of non-destructive or preparatory procedures can improve the design of such projects and, in some cases may eliminate the necessity for destructive sampling entirely (Stantis et al., 2025).

The utility of isotopic analysis in forensic anthropology is currently limited due to significant methodological challenges, such as a lack of analytical precision, insufficient reference materials, a lack of standardized operating procedures, and the presence of various confounding variables. In order to have validity and reproducibility of research findings, it is important that researchers have a thorough understanding of these analytical constraints and the destructive nature of the sampling process (Meier-Augenstein, 2010; Meier-Augenstein & Fraser, 2008). One of the main challenges is the significant isotopic overlap between geographically distinct places with comparable meteorological and environmental circumstances, complicating geographic provenance. Distinguishing between similar settings can be challenging due to modest isotopic difference indicated in parts per thousand. Furthermore, confounding factors such as food globalization, migration, and environmental contamination can transfer non-local isotope signatures into human tissues, interfering with isotopic signals (Bowen, 2010; Fraser et al., 2006; Sharp, 2017). Apart from those limitations associated with destructive sampling methods, the reliability of isotopic data is further limited by diagenesis. This refers to changes in skeletal material that are caused by interaction with the burial matrix. Such changes can fundamentally alter isotopic data. Therefore, rigorous screening methods are necessary to separate biological specimen from environmental contaminants (Bartelink & Chesson, 2019).

For example, incorporating multi-isotope analysis has become a standard approach in enhancing geographic data, thus providing a more comprehensive framework in understanding human migration in ancient times. By layering distinct isotopic systems, the intersection of various isoscapes narrows the likely location of habitation, enhances the accuracy of the identification. However, the effectiveness of this strategy is often limited by a global disparity in data availability. These shortages can be overcome by the systematic collection of reference data from underrepresented geographical regions of the world, which will be made available to the scientific community. Until such time that standardized methodologies, reference databases, and analytical instrumentation are perfected, isotopic analysis will likely remain a secondary line of evidence in archaeological reconstruction. Although isotope utility can help in gaining better understanding of the past, it cannot be denied that more protocols are required to further smoothened the process. Overcoming these obstacles would thus enhance the accuracy investigative utility of isotopic evidence (Bartelink & Chesson, 2019; Fraser et al., 2006; Weller, 2024).

Conclusion

In the course of history, people were subjected to the threats of several types of infectious diseases like tuberculosis, leprosy, treponematosis, and Justinian Plague. Isotope analysis proves to be an extremely important instrument that transforms the study of past epidemics. Analysis of chemical elements, such as oxygen, carbon, strontium and nitrogen, allows to go further and beyond pathogen identification and explore the intricate relation between the host and a disease. Such isotopes provide important information about diet changes, mobility and spread of the outbreak. The inclusion of isotopic data into the study of past pandemics does not only allow us to learn about past populations but also serves as a useful tool for modern public health issues. By mapping the patterns and how past populations navigated the stresses associated with epidemics help create an important framework for the behaviour of modern infectious diseases.

However, there are a lot of challenges involved when using this technique in bioarchaeology. The destructive nature of isotopic sampling means that the bioarchaeological materials need to be carefully. This highlights the conflict between getting high quality data and conserving precious specimens. To fully achieve the effectiveness of isotopic sampling in infectious disease studies, there is a necessity to underscore the development of improved sample collection methods. Resolving such challenges will go a long way in making isotopic analysis an integral part of epidemiological studies.

Conflict of Interest

Authors declare no conflict of interest.

Acknowledgements

We would like to thank Param Pujye Swami Ramdev Ji for his immense support and guidance. Authors highly acknowledge the help and support provided by Patanjali Research Institute & University of Patanjali, Haridwar, India.

REFERENCES

Anastasiou, E., & Mitchell, P. D. (2013). Palaeopathology and genes: Investigating the genetics of infectious diseases in excavated human skeletal remains and mummies from past populations. *Gene*, *528*(1), 33–40. https://doi.org/10.1016/j.gene.2013.06.017

Arriaza, B. T., Salo, W., Aufderheide, A. C., & Holcomb, T. A. (1995). Pre-Columbian tuberculosis in Northern Chile: Molecular and skeletal evidence. *American Journal of Physical Anthropology*, *98*(1), 37–45. https://doi.org/10.1002/ajpa.1330980104

Aufderheide, A. C., & Rodríguez Martín, C. (2011). *The Cambridge encyclopedia of human paleopathology* (First paperback edition). Cambridge University Press.

Barry, C. E., Boshoff, H. I., Dartois, V., Dick, T., Ehrt, S., Flynn, J., Schnappinger, D., Wilkinson, R. J., & Young, D. (2009). The spectrum of latent tuberculosis: Rethinking the biology and intervention strategies. *Nature Reviews Microbiology*, *7*(12), 845–855. https://doi.org/10.1038/nrmicro2236

Bartelink, E. J., & Chesson, L. A. (2019). Recent applications of isotope analysis to forensic anthropology. *Forensic Sciences Research*, *4*(1), 29–44. https://doi.org/10.1080/20961790.2018.1549527

Benedictow, O. J. (2008). *The Black Death, 1346-1353: The Complete History*. Boydell Press.

Bennett, B. H., Parker, D. L., & Robson, M. (2008). Leprosy: Steps Along the Journey of Eradication. *Public Health Reports®*, *123*(2), 198–205. https://doi.org/10.1177/003335490812300212

Bodamer, O. A. F. (2001). Uses of stable isotopes in clinical diagnosis and research in the paediatric population. *Archives of Disease in Childhood*, *84*(5), 444–448. https://doi.org/10.1136/adc.84.5.444

Bos, K. I., Schuenemann, V. J., Golding, G. B., Burbano, H. A., Waglechner, N., Coombes, B. K., McPhee, J. B., DeWitte, S. N., Meyer, M., Schmedes, S., Wood, J., Earn, D. J. D., Herring, D. A., Bauer, P., Poinar, H. N., & Krause, J. (2011). A draft genome of Yersinia pestis from victims of the Black Death. *Nature*, *478*(7370), 506–510. https://doi.org/10.1038/nature10549

Bowen, G. J. (2010). Isoscapes: Spatial Pattern in Isotopic Biogeochemistry. *Annual Review of Earth and Planetary Sciences*, *38*(1), 161–187. https://doi.org/10.1146/annurev-earth-040809-152429

Cohn, S. K. (2008). Epidemiology of the Black Death and Successive Waves of Plague. *Medical History*, *52*(S27), 74–100. https://doi.org/10.1017/S0025727300072100

Collins, D., & Powell, M. L. (2011). Treponematosis: Past, Present, and Future. In A. L. Grauer (Ed.), *A Companion to Paleopathology* (1st ed., pp. 472–491). Wiley. https://doi.org/10.1002/9781444345940.ch26

Economou, C., Kjellström, A., Lidén, K., & Panagopoulos, I. (2013). Ancient-DNA reveals an Asian type of Mycobacterium leprae in medieval Scandinavia. *Journal of Archaeological Science*, *40*(1), 465–470. https://doi.org/10.1016/j.jas.2012.07.005

Fornaciari, A. (2017). Environmental Microbial Forensics and Archaeology of Past Pandemics. *Microbiology Spectrum*, *5*(1), 5.1.26. https://doi.org/10.1128/microbiolspec.EMF-0011-2016

Fraser, I., Meier-Augenstein, W., & Kalin, R. M. (2006). The role of stable isotopes in human identification: A longitudinal study into the variability of isotopic signals in human hair and nails. *Rapid Communications in Mass Spectrometry*, *20*(7), 1109–1116. https://doi.org/10.1002/rcm.2424

Froes, L. A. R., Trindade, M. A. B., & Sotto, M. N. (2022). Immunology of leprosy. *International Reviews of Immunology*, *41*(2), 72–83. https://doi.org/10.1080/08830185.2020.1851370

Hackett, C. J. (1975). An introduction to diagnostic criteria of syphilis, treponarid and Yaws (treponematoses) in dry bones, and some implications. *Virchows Archiv A Pathological Anatomy and Histology*, *368*(3), 229–241. https://doi.org/10.1007/BF00432525

Henneberg, M., Holloway-Kew, K., & Lucas, T. (2021). Human major infections: Tuberculosis, treponematoses, leprosy—A paleopathological perspective of their evolution. *PLOS ONE*, *16*(2), e0243687. https://doi.org/10.1371/journal.pone.0243687

Hershkovitz, I., Donoghue, H. D., Minnikin, D. E., May, H., Lee, O. Y.-C., Feldman, M., Galili, E., Spigelman, M., Rothschild, B. M., & Bar-Gal, G. K. (2015). Tuberculosis origin: The Neolithic scenario. *Tuberculosis*, *95*, S122–S126. https://doi.org/10.1016/j.tube.2015.02.021

Katzenberg, M. A., & Lovell, N. C. (1999). Stable isotope variation in pathological bone. *International Journal of Osteoarchaeology*, *9*(5), 316–324. https://doi.org/10.1002/(SICI)1099-1212(199909/10)9:5%253C316::AID-OA500%253E3.0.CO;2-D

Larsen, C. S. (2018). The Bioarchaeology of Health Crisis. *Annual Review of Anthropology*, *47*, 295–313. JSTOR.

Larsen, C. S., & Crespo, F. (2022). Paleosyndemics: A Bioarchaeological and Biosocial Approach to Study Infectious Diseases in the Past. *Centaurus*, *64*(1), 181–196. https://doi.org/10.1484/J.CNT.5.130031

Lewis, C. M., Akinyi, M. Y., DeWitte, S. N., & Stone, A. C. (2023). Ancient pathogens provide a window into health and well-being. *Proceedings of the National Academy of Sciences*, *120*(4), e2209476119. https://doi.org/10.1073/pnas.2209476119

Macallan, D. C. (1999). Malnutrition in tuberculosis. *Diagnostic Microbiology and Infectious Disease*, *34*(2), 153–157. https://doi.org/10.1016/S0732-8893(99)00007-3

Meier-Augenstein, W. (2010). *Stable Isotope Forensics: An Introduction to the Forensic Application of Stable Isotope Analysis* (1st ed.). Wiley. https://doi.org/10.1002/9780470688762

Meier-Augenstein, W., & Fraser, I. (2008). Forensic isotope analysis leads to identification of a mutilated murder victim. *Science & Justice*, *48*(3), 153–159. https://doi.org/10.1016/j.scijus.2007.10.010

Mendum, T. A., Schuenemann, V. J., Roffey, S., Taylor, G., Wu, H., Singh, P., Tucker, K., Hinds, J., Cole, S. T., Kierzek, A. M., Nieselt, K., Krause, J., & Stewart, G. R. (2014). Mycobacterium leprae genomes from a British medieval leprosy hospital: Towards understanding an ancient epidemic. *BMC Genomics*, *15*(1), 270. https://doi.org/10.1186/1471-2164-15-270

Mora, A., Pacheco, A., Roberts, C. A., & Smith, C. (2021). Palaeopathology and amino acid δ13C analysis: Investigating pre-Columbian individuals with tuberculosis at Pica 8, northern Chile (1050-500 BP). *Journal of Archaeological Science*, *129*, 105367. https://doi.org/10.1016/j.jas.2021.105367

Mousavi-Sagharchi, S. M. A., Ghorbani, A., Meskini, M., & Siadat, S. D. (2025). Historical examination of tuberculosis; from ancient affliction to modern challenges. *Journal of Infection and Public Health*, *18*(3), 102649. https://doi.org/10.1016/j.jiph.2024.102649

North, J. (2013). The Death Toll of Justinian’s Plague and Its Effects on the Byzantine Empire. *Armstrong Undergraduate Journal of History*, *3*(1). https://doi.org/10.20429/aujh.2013.030101

Ortner, D. J. (Ed.). (2003). *Identification of Pathological Conditions in Human Skeletal Remains*. Elsevier. https://doi.org/10.1016/B978-0-12-528628-2.X5037-6

Panzer, S., Peschel, O., Haas-Gebhard, B., Bachmeier, B. E., Pusch, C. M., & Nerlich, A. G. (2014). Reconstructing the Life of an Unknown (ca. 500 Years-Old South American Inca) Mummy – Multidisciplinary Study of a Peruvian Inca Mummy Suggests Severe Chagas Disease and Ritual Homicide. *PLoS ONE*, *9*(2), e89528. https://doi.org/10.1371/journal.pone.0089528

Reitsema, L. J., & Holder, S. (2018). Stable Isotope Analysis and the Study of Human Stress, Disease, and Nutrition. *Bioarchaeology International*, *2*(2). https://doi.org/10.5744/bi.2018.1018

Robbins, G., Tripathy, V. M., Misra, V. N., Mohanty, R. K., Shinde, V. S., Gray, K. M., & Schug, M. D. (2009). Ancient Skeletal Evidence for Leprosy in India (2000 B.C.). *PLoS ONE*, *4*(5), e5669. https://doi.org/10.1371/journal.pone.0005669

Roberts, C. A., Millard, A. R., Nowell, G. M., Gröcke, D. R., Macpherson, C. G., Pearson, D. G., & Evans, D. H. (2013). Isotopic tracing of the impact of mobility on infectious disease: The origin of people with treponematosis buried in hull, England, in the late medieval period. *American Journal of Physical Anthropology*, *150*(2), 273–285. https://doi.org/10.1002/ajpa.22203

Roffey, S., Tucker, K., Filipek-Ogden, K., Montgomery, J., Cameron, J., O’Connell, T., Evans, J., Marter, P., & Taylor, G. M. (2017). Investigation of a Medieval Pilgrim Burial Excavated from the Leprosarium of St Mary Magdalen Winchester, UK. *PLOS Neglected Tropical Diseases*, *11*(1), e0005186. https://doi.org/10.1371/journal.pntd.0005186

Sakai, T., & Morimoto, Y. (2022). The History of Infectious Diseases and Medicine. *Pathogens*, *11*(10), 1147. https://doi.org/10.3390/pathogens11101147

Santacroce, L., Prete, R. D., Charitos, I. A., & Bottalico, L. (2021). Mycobacterium Leprae: A Historical Study on the Origins of Leprosy and Its Social Stigma. *Infezioni in Medicina*, *29*(4). https://doi.org/10.53854/liim-2904-18

Sharp, Z. (2017). *Principles of Stable Isotope Geochemistry, 2nd Edition*. https://doi.org/10.25844/H9Q1-0P82

Squires, K., Booth, T., & Roberts, C. A. (2019). The Ethics of Sampling Human Skeletal Remains for Destructive Analyses. In K. Squires, D. Errickson, & N. Márquez-Grant (Eds.), *Ethical Approaches to Human Remains* (pp. 265–297). Springer International Publishing. https://doi.org/10.1007/978-3-030-32926-6_12

Stantis, C., & Kendall, E. J. (2022). Isotopes in Paleopathology. In A. L. Grauer (Ed.), *The Routledge Handbook of Paleopathology* (1st ed.). Routledge. https://doi.org/10.4324/9781003130994

Stantis, C., Schaefer, B. J., Correia, M. A., Alaica, A. K., Huffer, D., Plomp, E., Di Giusto, M., Chidimuro, B., Rose, A. K., Nayak, A., & Kendall, E. J. (2025). Ethics and applications of isotope analysis in archaeology. *American Journal of Biological Anthropology*, *186*(1), e24992. https://doi.org/10.1002/ajpa.24992

Taylor, G. M., Tucker, K., Butler, R., Pike, A. W. G., Lewis, J., Roffey, S., Marter, P., Lee, O. Y.-C., Wu, H. H. T., Minnikin, D. E., Besra, G. S., Singh, P., Cole, S. T., & Stewart, G. R. (2013). Detection and Strain Typing of Ancient Mycobacterium leprae from a Medieval Leprosy Hospital. *PLoS ONE*, *8*(4), e62406. https://doi.org/10.1371/journal.pone.0062406

Tomei, S., D’Aurelio, A., Sakal, F., Castorina, F., Naimi, F. A., & Labarga, C. M. (2023). Ancient Biomolecules Unravel our History: A Technical Update with Examples from the Middle East. *Journal Of Anthropological And Archaeological Sciences*, *8*(3), 1096–1115.

Van Der Kuyl, A. C. (2022). Historic and Prehistoric Epidemics: An Overview of Sources Available for the Study of Ancient Pathogens. *Epidemiologia*, *3*(4), 443–464. https://doi.org/10.3390/epidemiologia3040034

Vlok, M., & Buckley, H. (2021). Paleoepidemiological Considerations of Mobility and Population Interaction in the Spread of Infectious Diseases in the Prehistoric Past. *Bioarchaeology International*, *6*(1–2), 77–107. https://doi.org/10.5744/bi.2020.0026

Vytlačil, Z., Durand, R., Kacki, S., Holleville, M., Drtikolová Kaupová, S., Brůžek, J., Castex, D., & Velemínský, P. (2024). Well supplied in life, set aside in death: A multi-isotope study of Justinian plague victims from SAINT-DOULCHARD (France, 7th–8th centuries AD). *American Journal of Biological Anthropology*, *185*(1), e25002. https://doi.org/10.1002/ajpa.25002

Wagner, D. M., Klunk, J., Harbeck, M., Devault, A., Waglechner, N., Sahl, J. W., Enk, J., Birdsell, D. N., Kuch, M., Lumibao, C., Poinar, D., Pearson, T., Fourment, M., Golding, B., Riehm, J. M., Earn, D. J. D., DeWitte, S., Rouillard, J.-M., Grupe, G., … Poinar, H. (2014). Yersinia pestis and the Plague of Justinian 541–543 AD: A genomic analysis. *The Lancet Infectious Diseases*, *14*(4), 319–326. https://doi.org/10.1016/S1473-3099(13)70323-2

Weller, K. (2024). The Limitations and Future of Isotope Analysis in Forensic Anthropology. *Themis: Research Journal of Justice Studies and Forensic Science*, *12*(1). https://doi.org/10.55917/2324-6561.1123

Zink, A. R., Grabner, W., Reischl, U., Wolf, H., & Nerlich, A. G. (2003). Molecular study on human tuberculosis in three geographically distinct and time delineated populations from ancient Egypt. *Epidemiology and Infection*, *130*(2), 239–249. https://doi.org/10.1017/S0950268802008257