High prevalence of cholesterol-rich atherosclerotic lesions in ancient mummies: A near-infrared spectroscopy study

Computed tomography has been used previously in mummies to detect arterial calcification, which is a marker of later-stage atherosclerosis. Here, using the novel approach of near-infrared spectroscopy, we detected cholesterol-rich atherosclerotic plaques in arterial samples from ancient mummies. In this proof-of-concept study, we are the first to noninvasively detect these earlier-stage lesions in mummies from different geographical areas, suggesting that atherosclerosis has been present in humans since ancient times. (Am Heart J 2019;216:113-116.)

Atherosclerosis is an ancient disease.[1] More than a century ago, atherosclerotic lesions were found in Egyptian mummies dating back to 1580 BC.[2] In autopsy studies of mummies from different geographical locations, calcified lesions have been identified in almost all arterial beds.[3] These calcified lesions were found in the famed mummies of Egyptian pharaohs[2] and in mummies discovered in Peru, the Aleutian Islands, the American Southwest, Europe, and Mongolia.[4,5]

In earlier studies, invasive dissection and autopsy methods were used to identify lesions. Later, noninvasive imaging with computed tomography (CT) was used to detect foci of calcification as a sign of advanced atherosclerotic lesions.[6,7] Nondestructive, noninvasive imaging modalities are preferred methods for studying atherosclerosis in mummies. The presence of vascular calcification as detected by CT, although not sine qua non for an atherosclerosis diagnosis, suggests the high likelihood of its presence. However, calcified lesions, usually found in advanced-stage atherosclerosis, have a high specificity for atherosclerosis but a low sensitivity for detecting atherosclerosis in the absence of plaque calcification.[8] Over the last 2 decades, pathological studies have shown that cholesterol-rich, thin-cap fibroatheromas are the culprit lesions for about two thirds of acute coronary syndrome cases, although a large portion of such high-risk plaques lack significant calcification.[8] Therefore, relying only on vascular calcification as a sign of atherosclerosis can underestimate the prevalence of atherosclerosis.

In this study, we used near-infrared spectroscopy to examine a series of vascular samples from ancient mummies for the presence of cholesterol-rich plaques. Near-infrared spectroscopy uses the molecular spectral signature of samples, which has been validated against pathologic studies of atherosclerotic samples.[9] This noninvasive imaging technique has been used extensively to detect cholesterol-rich atherosclerotic lesions in human and animal studies.[10]

Methods

We used near-infrared spectroscopy to determine the presence of cholesterol-rich plaques in arterial samples from 5 mummies dating from the late Chinchorro to the Greco-Roman period. The mummified arterial segments were provided by the late Dr Arthur C. Aufderheide (University of Minnesota School of Medicine, Duluth, MN) (Figure 1). The presumptive cause of death was pneumonia in 3 mummies and renal disease in 1 and was unknown in 1 case (Table I). Three mummies dated to 2000 BC, and one was from AD 350-1000. The mummies belonged to the later Chinchorro era, Cabuza, and Egypt Dakhla oasis. The Chinchorro and Cabuza mummies are from South America (parts of present-day Peru and Chile), and the Dakhla Oasis mummies are from ancient Egypt. The estimated age at death for these mummies was between 18 and 60 years (Table I).

A near-infrared spectroscopy probe with a scanning area of 78 mm^2 was placed over each sample. The near-infrared spectra were collected using an InfraAlyzer 500 spectrophotometer (Bran and Luebbe, Germany). The instrument uses a tungsten light source to generate near-infrared light, with a 300-mW maximum power, which provides 2-mm tissue penetration. Absorbance values were obtained as log (1/R) data from 1100 to 2200 nm at 10-nm intervals. Analytical software was written in Mathematica 3.0 (Wolfram Research, Inc, Champaign, IL), Matlab 5.1 (The Math Works, Inc), and Speakeasy IV Eta (Speakeasy Computing Corp, Chicago, IL). The Caldatas program was used for calibration. We performed 3 near-infrared scans on each segment. Principal component regression was used to analyze the smoothed, scatter-corrected data. This regression technique transforms many correlated variables into a new set of noncorrelated variables and reduces the dimensionality of a data set by linear transformation with principal components. The near-infrared diffuse

Figure 1

Tissue sample: abdominal aorta from a 55- to 60-year-old male from the Late Chinchorro era (around 2000 BC), with acute pneumonia as the presumptive cause of death.

Table I. Demographics and characteristics of mummies and the source of tissue samples for arterial blood vessels

Estimated age (y)	Sex	Arterial bed	Presumptive cause of death	Culture
18	Male	Thoracic aorta	Renal failure	Cabuza AD 350-1000
55-60	Male	Abdominal aorta	Pneumonia	Late Chinchorro 2000 BC
40	Female	Carotid artery	Pneumonia and emphysema	Cabuza AD 350-1000
35	Female	Abdominal aorta	Bronchopneumonia	Late Chinchorro 2000 BC
23	Male	Right subclavian artery	Unknown	Greco-Roman period Egypt Dakhla oasis 332 BC-AD 395

reflectance spectra of cholesterol have been previously identified and established by studying samples of crystalline cholesterol and normal arteries. (Figure 2, *A* and *B*).

To further examine sample calcification, we used a micro-CT scanner. The Explore Locus RS preclinical scanner (GE Medical System, Waukesha, WI) is a cone-beam volume CT system that uses a tungsten-source x-ray tube operating at 80 kV and 450 μA.

No extramural funding was used to support this work.

Results

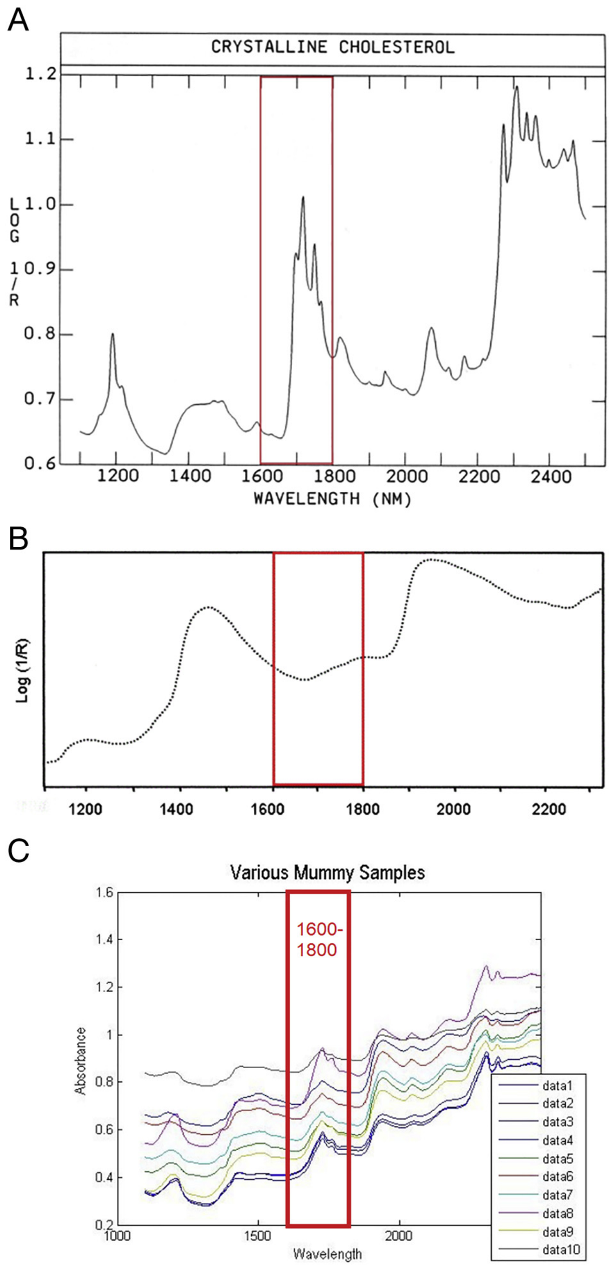
Cholesterol has absorption bands in the wavelength range of 1600 to 1800 nm. We identified cholesterol deposition in the arteries of all samples by inspecting an increased absorption signal in the 1600- to 1800-nm absorbance wavelengths on near-infrared spectroscopy; this finding is specific for cholesterol-rich plaque (atherosclerotic lesions) (Figure 2). We also found small degrees of calcification in the micro-CT images.

Discussion

Using near-infrared spectroscopy, we demonstrated the presence of cholesterol-rich atherosclerotic lesions in ancient mummies dating as far back as 2000 BC. In this proof-of-concept study, we have shown for the first time the feasibility of examining ancient samples with near-infrared spectroscopy, a noninvasive imaging modality that can be used to study atherosclerotic plaques. Near-infrared spectroscopy uses the near-infrared region of the electromagnetic spectrum (from about 700 to 2500 nm) and has been applied extensively in the noninvasive determination of the chemical composition of various sample types. A novel, catheter-based near-infrared spectroscopy imaging device has been approved for use in humans and studied in multiple clinical trials.[10]

Although initial autopsy studies and later CT studies have shown the presence of calcified atherosclerotic lesions in human mummies, our study offers new insight into the earlier pathological stages of atherosclerosis, identifying cholesterol-rich plaque as a hallmark of the

Figure 2



Near-infrared spectroscopy of crystalline cholesterol, normal artery, and ancient mummy arteries. **A**, Near-infrared absorption spectra of crystalline cholesterol (red box: wavelength range of 1600-1800 nm) showing a signal spike in the wavelength range of 1600 to 1800 nm, which determines increasing absorption in this range. **B**, Near-infrared absorption spectra of a normal artery without lipid-filled plaque (red box: lack of signal spike in the 1600- to 1800-nm range). **C**, Near-infrared absorption spectra of mummies' artery samples. Evidence of cholesterol deposition in 5 mummy artery samples (2 lines/sample) determined by an increased signal spike in the 1600- to 1800-nm absorbance wavelengths (red box area), which is specific for cholesterol-rich atherosclerotic plaques.

disease. We found these plaques in all samples, mainly without heavy calcification. Our findings suggest that cholesterol accumulation in arteries is not a disease only of modern times but may date to ancient times. Our samples were obtained from ordinary men and women, not from kings or pharaohs. They were probably active hunter-gatherers, and the South American subjects lived close to the ocean, presumably providing them with a diet rich in fish and vegetables. However, atherosclerosis was present in these populations, even at relatively young ages. One possible explanation for this finding is that regular exposure to smoke from a fire (for heating and cooking), along with the potential presence of multiple chronic bacterial and parasitic infections resulting in chronic latent inflammation, could have contributed to the development of atherosclerosis. This explanation supports the "response-to-injury" hypothesis for the origin of atherosclerosis. However, the effect of genetic factors in the development of atherosclerosis in these populations cannot be ignored. Apparently, an interplay of nature versus nurture has been in effect for thousands of years.

Our study is limited by the small sample size. Studying a larger number of samples from various geographical locations that have a wider background variation will allow us to further characterize the extent and nature of atherosclerosis. Finally, correlating these studies with presumptive histopathologic results will help confirm the imaging findings and provide better insight into the pathogenesis of atherosclerosis in ancient samples.

Conclusions

We found evidence of atherosclerosis in different vascular beds of mummy samples across various time periods and among different cultures, supporting previous findings.[7] Noninvasive near-infrared spectroscopy is a promising technique for studying ancient mummies of various cultures to gain insight into the origins of atherosclerosis. Near-infrared spectroscopy has the ability to detect cholesterol-rich atherosclerotic plaques. This noninvasive technique provides further information beyond CT scan studies, shows a higher prevalence of atherosclerosis, and may be useful in further identifying noncalcified, early-stage atherosclerotic plaques in ancient mummy samples.

Am Heart J 2019;113–116.
0002-8703
https://doi.org/10.1016/j.ahj.2019.06.018

Mohammad Madjid, MD, MS
Department of Medicine, McGovern Medical School, University of Texas Health Science Center at Houston, Houston, TX

Reprint requests: Mohammad Madjid, MD, Department of Medicine, McGovern Medical School, University of Texas Health Science Center at Houston, 6431 Fannin, MSB 1.246, Houston, TX 77030
E-mail: mmadjid@gmail.com

Payam Safavi-Naeini, MD
Electrophysiology Clinical Research & Innovations, Texas Heart Institute, Houston, TX

Robert Lodder, PhD
Department of Pharmaceutical Sciences, University of Kentucky, Lexington, KY, United States

References

1. Madjid M, Aboshady I, Casscells SW, et al. History of atherosclerosis and vulnerable plaque research. In: Virmani R, editor. Vulnerable Atherosclerotic Plaque. London: Blackwell Publishing; 2006. p. 3-17.
2. Ruffer MA. On arterial lesions found in Egyptian mummies (1580 B.C.-525 A.D.). J Pathol Bacteriol 1911;15:453-62.
3. Thomas GS, Wann LS, Narula J. What do mummies tell us about atherosclerosis? Glob Heart 2014;9:185-6.
4. Zimmerman MR, Yeatman GW, Sprinz H, et al. Examination of an Aleutian mummy. Bull N Y Acad Med 1971;47:80-103.
5. El-Najjar MY, Benitez J, Fry G, et al. Autopsies on two native American Mummies. Am J Phys Anthropol 1980;53:197-202.
6. Allam AH, Thompson RC, Wann LS, et al. Computed tomographic assessment of atherosclerosis in ancient Egyptian mummies. JAMA 2009;302:2091-4.
7. Thompson RC, Allam AH, Lombardi GP, et al. Atherosclerosis across 4000 years of human history: the Horus study of four ancient populations. Lancet 2013;381:1211-22.
8. Falk E, Nakano M, Bentzon JF, et al. Update on acute coronary syndromes: the pathologists' view. Eur Heart J 2013;34:719-28.
9. Stone GW, Maehara A, Muller JE, et al. Plaque characterization to inform the prediction and prevention of periprocedural myocardial infarction during percutaneous coronary intervention: the CANARY Trial (Coronary Assessment by Near-infrared of Atherosclerotic Rupture-prone Yellow). JACC Cardiovasc Interv 2015;8:927-36.
10. Madder RD, Puri R, Muller JE, et al. Confirmation of the intracoronary near-infrared spectroscopy threshold of lipid-rich plaques that underlie ST-segment-elevation myocardial infarction. Arterioscler Thromb Vasc Biol 2016;36:1010-5.