

Forensic analysis reveals acute decompensation of chronic heart failure in a 3,500 years old Egyptian dignitary

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ABSTRACT (150 Words)

This journal uses unstructured abstracts; however, the abstract should include the following – background, brief description of methods and results (give specific data and their statistical significance, if possible), and conclusions. Emphasize new and important aspects of the study or observations.

Naturally preserved and embalmed bodies from archaeological contexts represent a powerful source of information for forensic investigators, especially in the fields of anthropology and pathology. Due to the preservation of 'soft and hard tissues', these ancient corpses often allow identification of pathological disease and, eventually, cause of death (either natural or violent). Also archaeological specimens enable forensic anthropologists to enhance their diagnostic methodology and improve the analysis of altered remains (1).

Here, we report on a possible case of acute decompensation of chronic left heart failure in a 3,500 year old Egyptian dignitary named Nebiri, described as "Chief of Stables" under the reign of Thutmoses III (1479-1424 BC; 18th Dynasty).

His plundered tomb (QV30) was discovered by E. Schiaparelli in 1904 in Luxor (2). Only the head (S.5109) (**Figure 1a**) and the canopic jars containing the internal organs- lung, stomach, liver and intestines- (S.5110, S.5111/02, S.5112, S.5113) were preserved and are currently housed at Turin's Fondazione Museo delle Antichità Egizie. Since the jar inscribed for Hapy (S.5111/02), the « guardian of the lungs », was partly broken (**Figure 1b**), direct access to organ sampling was allowed.

Methods

Prior to any further destructive analyses and in order to determine age and sex as well as to identify any eventual intra-vascular deposit within the carotid arteries (3), the complete head underwent a Multidetector Computed Tomography (MD-CT Phillips Brilliance 16 slices, slice thickness of 0.4 mms). To confirm tissue identification and verify the presence of any pulmonary pathological alterations, histology was carried out on a 1cm x 1cm lung biopsy following established protocols (4). In parallel, shotgun metagenomics was performed. DNA was extracted from another 1cm x 1cm lung biopsy, converted into Illumina libraries and sequenced to verify the presence of lung parasitic infection signatures (5-6).

Results

According to anthropological criteria, Nebiri was a middle aged (45 to 60 years old) male, and affected by severe periodontal disease with massive dental abscesses identified both in the upper and lower jaws (**Table 1**) (7). All the visualized maxillary teeth have lost their crowns, presumably through occlusal wear and carious lesions, and the pulp chambers are exposed creating a convenient pathway for the oral pathogens to access the root apex and to develop infection of the alveolar bone (**Figure 2a, b**).

Nebiri's head underwent partial trans-nasal excerebration during the embalming. A thin portion of the falx and the dura mater are still present, but retracted away from the cranial vault, without any visible pathological lesion. Portions of the cerebral and cerebellar hemispheres are preserved posteriorly (**Figure 1c,c1**). Some tiny, though evident, deposits of calcium were observed in the right internal carotid artery whereas no calcification was seen in the contralateral artery (**Figure 1d,d1**).

Although some diagenetic alterations due to the embalming and post-mortem dehydration processes could be observed, the typical lung's alveolar structure had been preserved and showed major pathological conditions: aggregates of siderophages - the so called "heart-failure" cells - (**Figure 1e, e1**) and a massive distention of the alveoli with residues of intra-alveolar hemorrhagic oedema (**Figure 1f, f1**). The presence of siderophages indicates that bleeding occurred more than 72 hours before death (8). The pulmonary bleeding associated with the cardiogenic pulmonary oedema we observed in Nebiri's lung seems to fit the classic signs of cardiovascular disease seen in modern patients (9). Evidence of simple focal anthracosis, a reflection of poor ventilation in rooms with open hearths, was also shown (10).

Histochemical staining (Ziehl-Neelsen stain, Periodic acid Schiffs-stain and Giemsa stain) did not show signs of tuberculosis, granulomas nor acid fast bacilli or other lung parasitoses. No ancient DNA signatures of Mycobacteria or of any other pathogenic bacterial species were identified by shotgun metagenomics.

Discussion

Our findings suggest that a pulmonary bleeding, possibly recurrent mini-bleedings, occurred at least several hours before Nebiri's death. Accordingly, at first hand, several other causes of pulmonary bleeding may be posited [e.g. leukemia, lung contusion (trauma), tumor, infection (fungal or parasite host, etc.), mitral valve stenosis, sarcoidosis, auto-immune diseases (such as Wegener disease or lupus); although these last two can be excluded mainly due to the lack of subsequent fibrosis]; however, due to the general status of this individual (sex, age, arterial calcifications), a diagnosis of cardiogenic insufficiency with recurrent mini-bleedings (chronic cardiac insufficiency) along with acute failure as shown by the massive intra-alveolar oedema seems the most logical.

Previous findings showed that atherosclerosis was not infrequent among middle-aged (45.1 ± 9.2 years) ancient Egyptians who lived over a time span of more than 2,000 years (3). In our case study, a middle-aged male presented with mild carotid atherosclerosis. In absence of the rest of the corpse, a full evaluation of the extent of calcifications in other artery walls (e.g. coronary, aortic, iliac and peripheral vascular beds) could not be performed. Similarly, the absence of the trunk did not allow investigating the morphology of the heart. Nevertheless, a previous study of CT scanned embalmed Egyptian human mummies showed that the common feature of dessication is to make the heart smaller and contracted. Only in one Third Intermediate Period (1064-656 BC) mummy out of the sixty analyzed individuals, was a large heart with a massive ventricle, possibly consistent with a cardiac pathology, identified (11).

Conversely, based on major histo-pathological findings, we present a collection of evidence which allows us to hypothesize that acute decompensation complicating chronic cardiac insufficiency was the likely cause of Nebiri's death. The underlying causes for this failure (e.g. coronary artery disease, cardiomyopathy, valvular disease, metabolic disorders of the heart muscle or chronic hypertension) remain unknown although chronic hypertension appears to be the best candidate.

The presence of inflammation due to the massive dental infection might also have played a role in the genesis of the cardiovascular disease (CDV) and the development of atherosclerotic plaques. A growing body of literature implicates infection, local and/or systemic inflammation due to chronic

periodontitis to have a role in the pathogenesis of atherosclerosis and an association between severe periodontal disease and increased risk for atherosclerosis, cardiovascular diseases and stroke through inflammatory pathways has been discussed over the last twenty years. Inflammation appears to play a role in the pathogenesis of the above pathologies although the exact mechanisms leading to the disease's phenotypic expression have yet to be fully understood (12-14).

Chronic left heart failure (CHF) is a frequent consequence of chronic heart disease. Currently, 20 million people worldwide are affected by CHF, they are mainly over 65 years old and have a poor prognosis (9). Our findings represent the oldest ever case of chronic heart failure yet to be discovered. Three other cases of pulmonary bleeding and intra-alveolar edema are reported in the literature (4, 15-16). However, the underlying causes for those deaths- a parasitic infestation (4) and two tubercular infections (15-16) were totally different from ours.

Further systematic analysis of canopic jar content will help clarifying whether this condition was rife in our ancestors or if its prevalence progressively increased in modern times. In parallel, this microscopic, genetic and radiological presentation may help forensic pathologists in the retrospective diagnosis of the cause of death when altered bodies are examined.

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Figure captions

Figure 1. a= Nebiri’s head; b= the Hapi canopic jar; c= CT scan sagittal right side; c1= CT scan sagittal left side; d= CT scan with focal atherosclerosis in the inner right carotid (arrow); d1= CT scan of the opposite left inner carotid without atherosclerosis; e= Photomicrographs of a histological section of Nebiri’s lung showing significant anthracosis and focal aggregates of siderophages (asterisk; Prussian blue stain, original magnification x600); e1= For comparison a recent case with CHF similar features of “heart failure” cells and anthracosis is shown (asterisk; Prussian blue stain, original magnification x600); f= Further lung photomicrographs with massive intra-alveolar edema in Nebiri’s lung (asterisk; H&E stain, original magnification x600); f1= For comparison a recent case with significant intra-alveolar edema is shown (asterisk; H&E stain, original magnification x600).

Figure 2. a= CT scan of Nebiri’s upper jaw shows the presence of eight peri-apical abscesses; b= CT scan of the lower jaw indicates the presence of two periapical abscesses.