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Articoli/*Articles*

HISTOPATHOLOGICAL EVIDENCE OF COAL-WORKERS' ANTHRACOFIBROSIS IN NATURAL MUMMIES OF 16TH-18TH CENTURIES

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SUMMARY

ANTHRACOFIBROSIS IN MUMMIES

During the restoration works of the Conversion of St. Paul Church in Roccapelago (Modena, Italy, 2009), a burial crypt containing the remains of about 400 individuals was discovered. Natural mummification has occurred in 60 cases. Our Research Group, focused the paleopathological investigation on the pulmonary tract, since the reported massive coal dust exposure of Roccapelago's dwellers even in parish records.
In 24 mummies, the alleged pulmonary tissue was biopsied through pre-existing solutions of continuity of the rib cage. In 18 cases (75%), lung tissue was recognized. In 7 cases (29,16%) we observed a massive deposition of black pigment, often nodular shaped, surrounded by dense fibrous tissue more evident with the Masson Trichrome staining. The morphological findings appeared diagnostics for anthracofibrosis secondary to massive pulmonary anthracosis.

Key words: Anthracofibrosis - Coal Worker - Resin Embedding - Natural Mummies

To our knowledge, this series is one of the most conspicuous paleopathological records of occupational lung diseases.

Introduction

Anthracosis is an old disease, reported even in mummies[1,2]. The term defines the black discoloration of the tracheobronchial tree due to the deposition of carbon, silica and quartz particles[3]. Globally, the prevalence of simple anthracosis condition ranges between 3.4% and 21%, mainly incidentally diagnosed during bronchoscopy. Less frequently (with a prevalence of 5.7% as cumulated mean), it can be associated with bronchial anthracofibrosis (BAF) with bronchial lumen occlusion and a chronic course similar to chronic obstructive pulmonary disease (COPD)[4,5]. Histologically, simple anthracosis shows a normal epithelial lining and carbon-like particles both inside the bronchial macrophages and in mediastinal lymph nodes. Bronchial fibrosis and reactive hyperplasia suggest the diagnosis of BAF[6,7,8].

In 2009, during the restoration work of the Conversion of St. Paul Church in Roccapelago (Modena, Italy), about 400 inhumed bodies were found in the basement, 60 of which in state of mummification. The Church was built in the last 15th century on the ruins of a fortress, hence the unusual architecture of the burial crypt (Fig. 1). Scientific investigations were approved on the burial site, involving several areas of research, such as archaeology, anthropology, paleopathology, entomology and ancient DNA analysis[9,10,11,12,13,14,15].

Our Research Group has focused on pulmonary tract, since the parish records described a widespread employment of the village dwellers as coal workers[16].

In this article, we report the histological results of the pulmonary biopsies on 24 mummies using two different tissue-embedding techniques.

Fig. 1. Loopholes in the wall of the crypt allowing a proper ventilation of the burial site.

Methods

Out of the total of 60 mummies we considered only 24 of them, namely the most superficial buried ones and those with pre-existing solutions of continuity of the rib cage through which we practiced biopsies of alleged pulmonary tissue. For each case, we divided the specimen into a smaller and a greater fragment. The first one was included in resin while the second was paraffin-embedded. We stained the histological sections of resin-embedded samples with Hematoxylin & Eosin, Perls, Masson trichrome, PAS and Von Kossa stains and the paraffin-embedded only with Hematoxylin & Eosin.

Resin-embedding technique

Technovit 8100 is a HEMA-based (hydroxyethyl methacrylate) plastic-embedding system for studies with light microscopy. Here we show the method modified for ancient tissues.

The specimen is fixed in absolute alcohol for 1 week. The dehydrated sample is then infiltrated with solution A (100ml of Technovit 8100 + 0.6 gr of Hardener I) and in-vacuum preserved (4°C) for 1 week. Therefore, the specimen is embedded in cavities of a Teflon plate (Histoform S: Technovit 7100/8100 10 cavities Dimensions: B x H x T: approx. 10 x 16 x 6.5 mm) using solution A + Hardener II (respectively 1.5 ml and 50 μl for each hole) to obtain the polymerization. After 24h (refrigerator 4C°), the Histoblock is put on the cavities and filled with the Technovit 3040 (a quickly hardening 2-component plastic with a methyl meth acrylate base). After 5-10 minutes, the histoblock (steady linked to the sample) is removed from the Teflon plate. The resin-embedded sample is now ready to be cut with rotary microtome determining semi-thin 2-3 μm thick slices.

Paraffin-embedding technique

The paraffin-embedding technique we used for mummies does not substantially differ from the routinely process for normal surgical specimens. An important difference with resin technique is the need of rehydration of the sample before processing. For rehydration we used the Sandison method (Stain Technol 1955) characterized by a gradual introduction of aqueous solutions. No formalin fixation is needed before the processing. Finally the paraffin-embedded sample is cut with rotary microtome, obtaining 5 μm thick slices.

Results

In 12 cases the right hemithorax was biopsied, in 6 cases the left hemithorax, in 4 cases the paracervical tissue, in one case the abdomen and in another case the paratracheal tissue.

In 18 cases (75%), it was possible to recognize lung tissue. Regarding the remaining 6 cases: in 2 cases the original tissue could not be identified, in other 2 cases only fibrous tissue was detected, while in the remaining two cases the sample was heavily contaminated by post

vital larvae or spores (PAS staining positive). In 6 cases (29% of the verified lung samples) a massive intratissutal deposition of black pigmentation (Fig. 2) was observed, associated with dense fibrosis which was highlighted by Masson Trichrome staining (Fig. 3). These findings agreed with the diagnosis of pulmonary anthracofibrosis. Moreover, in one case, we found a pigmentation intensely Perls staining positive (Fig. 4), suggesting previous bleeding due to a more severe form of pneumoconiosis anthracotica.

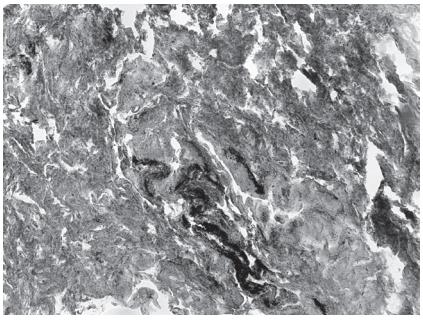

Fig. 2. Black pigmentation in pulmonary biopsy stained with Hematoxilin and Eosin.

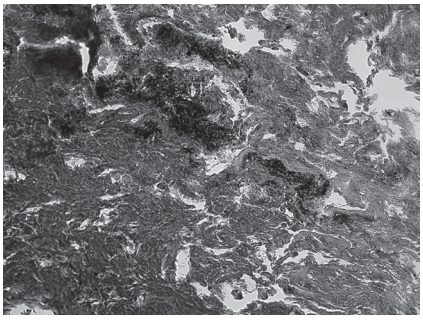

Fig. 3. Masson Trichrome stain revealing diffuse pulmonary fibrosis.

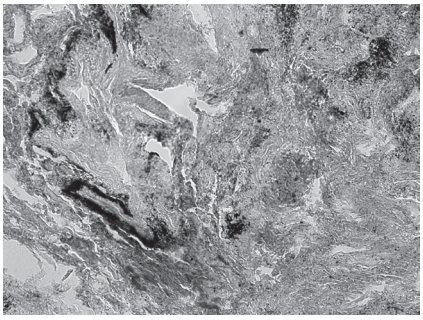

Fig. 4. Perls stain revealing hemosiderin pigmentation.

Discussion

In our project, we have been inspired by the pioneering work of the father of Occupational Medicine Bernardino Ramazzini (Carpi, 1633 – Padua, 1704), author of the masterpiece *De Morbis Artificum Diatriba*, where the severe pulmonary alterations secondary to work-related air pollution exposure were accurately described. As previously reported by Professor Alberto A. Bisetti in his pivotal works[17],

Ramazzini explained how workers in coalmines might suffer of respiratory diseases related to mineral exhalation: "Miners who maintain an almost daily contact with evil powders in the earth's depths… have lungs which absorb mineral exhalation and must be the first to suffer the attack of poisonous fumes"[18].

We carried out a paleopathological investigation on natural mummies, focusing on lung tissue, since their reported occupational dust exposure as coal workers. Therefore, we show a case of natural mummification of sixty individuals (dated 17th-18th centuries), allowed by the unusual microenvironment of the burial site. Basically, two holes in the wall of the crypt (loopholes used in medieval period to spot enemies) provided a dry and windy climate, which was optimal for tissue dehydration[19]. The process of mummification has been preceded by massive autolysis and focal putrefaction phase, especially splanchnic. The quite good state of conservation of mummies allowed us to investigate the pulmonary tract. For each case, we took a smaller and a greater pulmonary sample. The first one was resin-included while the second was paraffin-embedded. Thinner and more uniform slices were obtained using the first technique, improving the quality of microscopic image. Moreover, resin reduced the tissue coarctation, the main issue in handling ancient biological tissue. In addition, the slices could be easily histochemically stained without removing the resin, since the glycol-methacrylate is soluble in the most common colorants used. Another advantage was its compatibility with the immunohistochemical stainings, conferring an optimal identification of tissue antigen. Nevertheless, the incompatibility with greater samples (>2x2 mm) and the technical time lengthening represent the main challenges in routine practice.

We examined 24 cases, recognizing lung tissue in 18 of them (75%): 7 (about 1/3 of the study series) showed signs of severe pneumoconiosis secondary to pulmonary anthracosis. Although its ancient history (it was even discovered in mummies), the etiology of anthraco-

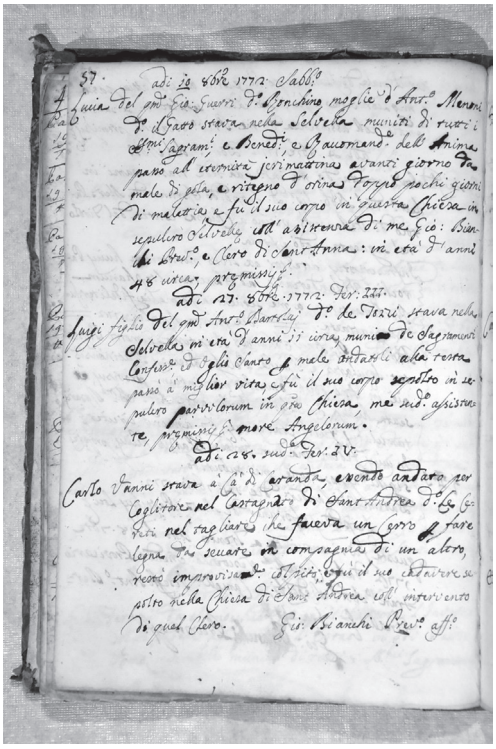

Fig. 5. Example of parish record regarding a working-related injury in cutting logs.

sis has not been completely revealed. It has been traditionally linked to coal dust inhalation, but also other exposures have been correlated with the disease (e.g. silica, aluminum, iron, biomass smoke)[20]. In our cases, the location and the parish records suggested a strong link with the coal-relating work. The Roccapelagos's dwellers used to obtain carbon piling one meter-sized logs in a conic shaped structure with a central fireplace. The bonfire was covered by dirt and leaves to avoid the direct contact between wood and air. The fire could burn even two weeks, continuously supervised, provoking a massive coal dust exposure (Fig. 5).

Conclusion

We show an example of natural mummification of inhumed individuals mainly due to the unusual architecture of the burial site, which allowed an appropriate ventilation of the crypt. The resin-embedding technique of the pulmonary biopsies brought good results, finally determining high definition slides for light microscopy, despite the antiquity of the biological tissues. Microscopically the finding of pulmonary anthracofibrosis (in about one third of the pulmonary biopsies) confirmed what subscribed in the parish records regarding the coal dust exposure of the Roccapelago's dwellers.

BIBLIOGRAPHY AND NOTES

1. Petrányi G, Anthracosis in members of the aristocracy and mummies in Hungary. Orv Hetil 1997;138(13):826.
2. Montgomerie R, The structural and elemental composition of inhaled particles in ancient Egyptian mummified lungs. Manchester, UK: The University of Manchester; 2013.
3. Tutluer S, Tanriover MD, Emri S, Systemic glucocorticoid and anti-tuberculosis therapy in a patient with coexisting tuberculosis and anthracosis. Sarcoidosis Vasc Diffuse Lung Dis. 2013;30(4):308-11.
4. Sigari N, Mohammadi S, Anthracosis and anthracofibrosis. Saudi Med J 2009;30(8):1063- 6.
5. Mirsadraee M, Saeedi P, Anthracosis of lung; Evaluation of potential causes. Journal of bronchology & Interventional Pulmonology 2005;12:84-7.
6. Na JO, Lim CM, Lee SD, Koh YS, Kim WS, Kim DS, Shim TS, Detection of Mycobacterium Tuberculosis in Bronchial Specimens Using a Polymerase Chain Reaction in Patients with Bronchial Anthracofibrosis. Tuberc Respir Dis 2002;53(2):161-172.
7. Amoli K, Bronchopulmonary disease in Iranian housewives chronically exposed to indoor smoke. Eur Respir J 1998;11(3):659-63.
8. Cheng NM, Yeh TW, Ho KC, Ng SH, Hsueh C, Yen TC, Liao CT. False positive F-18 FDG PET/CT in neck and mediastinum lymph nodes due to anthracosis in a buccal cancer patient. Clin Nucl Med 2011;36(10):963-4.

9. Cilli E, De Filippo C, Albanese D, Lugli F, Sordo M, Viola MF, Traversari M, Catalano G, Serventi P, De Fanti S, Quagliariello A, Labate D, Cipriani A, Luiselli D, Gruppioni G, Discovering past gut microbiomes through NGS analysis: the mummies of Roccapelago (MO). 21° Congresso dell'Associazione Antropologica Italiana: towards a next-generation anthropology: challenges & synergies. Bologna and Ravenna. 3-5 September 2015.
10. Traversari M, Milani V, Le mummie di Roccapelago: il progetto di musealizzazione come modello etico e scientifico. Pagani e Cristiani. Forme e attestazioni di religiosità del mondo antico in Emilia 2011;XI:181-184.
11. Traversari M, Figus C, Vazzana A, Gruppioni G, Galassi FM, Vellone VG, Fulcheri E, Neonatal and postnatal mortality in Roccapelago through the study of parish records and histological evidence. 7° Congresso Triennale di Anatomia Patologica; SIAPEC-IAP 2016, Pathologica 2016;108(4): 248-249.
12. Traversari M, Milani V, Osservazioni preliminari sul campione osteologico osservato. Le mummie di Roccapelago (XVI–XVIII sec.): vita e morte di una piccola comunità dell'appennino modenese. Archeologia e antropologia: una ricerca interdisciplinare. Pievepelago: 2016. pp. 157-161.
13. Petrella E, Piciucchi S, Feletti F, Barone D, Piraccini A, Minghetti C, Gruppioni G, Poletti V, Bertocco M, Traversari M, CT scan of thirteen natural mummies dating back to the XVI–XVIII centuries: an emerging tool to investigate living conditions and diseases in history. PLoS One 2016;11(6):e0154349.
14. Figus C, Traversari M, Scalise ML, Oxilia G, Vazzana A, Buti L, Sorrentini R, Gruppioni G, Benazzi S, The study of commingled non-adult human remains: Insights from the 16th–18th centuries community of Roccapelago (Italy). Journal of Archaeological Science Reports 2017;14:382-391.
15. Cilli E, De Fanti S, Quagliariello A, Sarno S, Serventi P, Traversari M, Luiselli D, Gruppioni G, Genetic analysis of the population of Roccapelago - Modena (Italy) (16th–18th C.) ArchaeoAnalytics. Chromatography and DNA Analysis in Archaeology, NPrint, Esposende 2015; 247-254.
16. Vellone VG, Repetto G, Traversari M, Vazzana A, Boano R, Gruppioni G, Fulcheri E. Pulmonary antracosis on natural mummies of XVI-XVIII century AD from Roccapelago (MO, Italy). Pathologica 2015;107(3-4):213-214.
17. Bisetti AA, Bernardino Ramazzini and occupational lung medicine. Ann N Y Acad Sci. 1988;534:1029-37.
18. Ramazzini B, De morbis artificum diatriba. A.Capponi, Ed. Modena, 1700. G. Van de Water Ed. Utrecht, 1703 (IInd ed.). G.B. Conzetto Ed. Padova, 1713 (IIIrd ed.).

19. Traversari M, Feletti F, Vazzana A, Gruppioni G, Frelat MA, Three cases of developmental dysplasia of the hip on partially mummified human remains (Roccapelago, Modena, 18th Century): a study of palaeopathological indicators through direct analysis and 3D virtual models. Bull Mém Soc Anthropol Paris 2016;28:202-212.
20. Walker R, Parsche F, Bierbrier M, McKerrow JH, Tissue identification and histologic study of six lung specimens from Egyptian mummies. Am J Phys Anthropol 1987;72(1):43-8.

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