HAEMOLYTIC ANEMIA IN TWO 500-YEAR-OLD SKULLS FROM THE CARDAMOMS MOUNTAINS, CAMBODIA: PALEORADIOLOGY STUDY

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Abstract

Two 500 year-old skulls, with possible signs of thalassemia, were selected from a burial jar site located in the Cardamoms mountains in Southwest Cambodia for a multidisciplinary investigation. This study includes physical and radiological examinations. The prevalence of thalassemia is high in the extant population of the Southeast-Asian mainland. We report the radiological findings, which include both plain films and computed tomography (CT) and discuss the role of radiology in the diagnosis of thalassemia in ancient bones.

Introduction

Legend has it that the Cardamoms forest may have served as an important place for people to hide, particularly for the royal family after the invasion of Loevek city by the Siamese in 1593 CE. No evidence to support this theory has yet been found, however the discovery of burial jars sites by Jean Ellul, French ethnologist in the 1960s may help determine the significance of this forest[1]. Bioarchaeological analysis of the skeletons obtained therein may give insight into how people may have survived in an area where malaria is endemic. Therefore the purpose of this paper is to describe

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[1] Martin 1997.

the radiological findings in two 500-year-old skulls that were suggestive of a chronic hemolytic anemia, most likely thalassemia. The pathogenesis of thalassemia in the ancient population living in the malaria-infested Cardamom Mountains in Cambodia will be discussed.

Materials and Methods

1. Skeletons and archaeological context

In March 2000, under the Cardamom Conservation Program working with the department of Forestry and Wildlife and the Agriculture Forestry and Fisheries ministry, 15 post-Angkorian burial jar sites were re-discovered within the Cardamoms forest of Southwest Cambodia. In February 2003, a multidisciplinary team headed by the senior author (RC) was sent to the malaria-infested Cardamoms forest to investigate the burial jar site near Phum Roleak Kang Cheung village. The expedition was supported by funds from the National Geographic Channel, Australia, for which a documentary film was produced (Becker Entertainment). Forty jars were found. Two of the most intact of these jars were brought to Phnom-Penh for further analysis.

One jar contained four individuals, another three; with a total of one child and six adults. Of the adults, five were female aged 18-25 years old.

2. Radiological analysis

X-ray analysis was performed in the Radiology Department of Calmette Hospital in Phnom-Penh. Radiographs of skulls were obtained using standard X-ray machine.

CT protocols: Two skulls with cribra orbitalia underwent a radiological investigation using both conventional X-rays and CT scan. CT (Toshiba, Auklet, Japan) was performed using the following technical parameters: 120kV, 200mAs, 5mm thickness, 10mm skip and 1 second scan time per rotation. Multiplanar reconstruction was performed to convert the acquired axial plane into coronal plane. The data was saved on a CD and selected images were printed on hardcopy film for interpretation.

3. Biomolecular studies were not conducted because they are not available in Cambodia, and exporting the skeletal materials was not allowed by the Cambodian authorities.

Results

Analysis of the ceramics from the burial jars site revealed three different origins. One is typical for Siamese, another middle Ming period Chinese and the third resembles known Siamese wares but is of uncertain origin. The ceramic techniques date to a period from the 14th to 15th centuries. Carbon14 dating of the human remains within supports this placing them in the 1430-1480 CE range. Despite the fact that these remains are 100 years older than would be expected of the fleeing royal family, given the multiple origins of these ceramics it is likely that the people were at least important enough to have had access to external trade. On gross inspection two of the six adults showed cribiform changes on the roof of the orbit known as cribra orbitalia.

Radiographic findings in both skulls include a diffuse calvarial thickening. This was associated with a "hair-on-end" pattern in one skull and a trabecular coarsening of the diploic space on the other. The "hair-on-end" pattern was identified in the parietal bones. The frontal sinuses of the skull displaying this pattern were small. They were normal in size in the latter skull.

CT confirmed the thickening of the calvarium but did not clearly demonstrate the "hair-on-end" pattern or trabecular coarsening. In one skull the maxillary sinuses were small, with thickening of the sinus wall.

Discussion

The radiological findings described in these specimens have been recognized by paleo-pathologists in previous skeletal remains under the term porotic hyperostosis, and are well known to radiologists as suggesting a chronic hemolytic anemia in extant populations[2,3,4,5,6,7].

In our case, the "hair-on-end" pattern involves the parietal bone, which is the most common location for porotic hyperostosis[5], In modern patients these changes may result from bone marrow hyperplasia in the diploic space. In severe and chronic anemia the outer table is perforated by proliferating marrow, which undermines and uplifts the periosteum developing bony spicules that give rise to the "hair-on-end" pattern[8]. Of the anemias, changes are most pronounced in thalassemia major[4].

[2] Agarwal, Dhar, Shah, Bhardwaj 1970.
[3] Aksoy, Camli, Erdem 1966.
[4] Mosely 1974.
[5] Ponec and Resnick 1984.
[6] Sebes and Diggs 1979.
[7] Steinbock 1976.
[8] Hart 1981.

Our specimens demonstrated diploic thickening, which is seen in 22% of patients with porotic hyperostosis[9]. There was also a diffuse thinning of the outer table, with focal areas that completely lacked outer cortex. This pattern is seen in between 20 to 90% of cases of clinical chronic anemia[9]. The coarse trabeculae seen in one skull are common according to Stuart-Macadam, and are present in 69% of cases of porotic hyperostosis.

Neither of our two cases had radiological orbital rim changes, known as cribra orbitalia. Due to the limited research time available at Calmette Hospital, our protocol did not include thin cuts in the coronal plane that would have shown orbital rim changes. However, the images that were obtained carried enough information to allow an accurate diagnosis. Since the time of our investigation, Exner has described a spiral CT and post-processing protocol for demonstrating cribra orbitalia[10], which is otherwise seen on physical examination. The presence of small maxillary sinuses in one skull also indicates a medullary hyperplasia secondary to chronic hemolytic anemias because they cause inhibition of sinus development[11]. Patients with thalassemia major may develop characteristic facial features, whereas widening of the diploic space may be the only skull manifestation in thalassemia minor[4,12]. The facial bones and paranasal sinuses are rarely involved in other types of anemias. Paleopathologists have long described these morphological findings in skeletal remains; a thickened diploic space and direct visualization of coarsened trabeculae due to loss of overlying cortex symmetrically involving the parietal and frontal bones, more often than the occipital bones 13. In early and minor forms of porotic hyperostosis outer cortex resorption may not be present. Cribra orbitalia has similar morphologies and demographics to porotic hyperostosis and may be the result of the same pathology. Paranasal sinus involvement is particularly characteristic in thalassemia[13]. The radiographic examination of ancient skulls reveals findings concordant with findings in modern patients with anemia supporting the theory that this radiographic pattern is equivalent to porotic hyperostosis8. Furthermore, this suggests that at least one of the mechanisms behind the development of porotic hyperostosis may be anemias. "Hair-on-end" and trabecular thickening identified in these two specimens only suggest a differential diagnosis that would include thalassemia, other congenital hemolytic anemias, iron deficiency anemia, congenital cyanotic heart disease and polychythemia vera[8,14]. Porotic hyperostosis has not only been attributed

[9] Stuart-MacAdam 1987.
[10] Exner, Boqusch, Sokiranski 2004.
[11] Reimann, Kayhan, Talati, Gokmen 1975.
[12] Logothetis, Economidou, Constantoulakis, Augoustaki, Swenson, Bilek 1971.
[13] Auferheide and Rodriguez-Martin 19980.
[14] Daly 1987.

to these diseases but to many to many other factors which include geographic distribution, sex, diet and pathogen load[8,15].

Pathogen load may play a complex role in marrow hyperplasia. It has been proposed that a hypoferric state may be an adaptive mechanism for living with high pathogen load, which may in turn predispose to iron deficiency anemia and development of porotic hyperostosis[15]. Fish tapeworm (Diphyllobothrium) may cause vitamin B12 depletion and ineffective erythropoisis leading to hyperplastic marrow suggesting a correlation with fish consumption habits[16]. Chronic malaria may also lead to anemias[15,17]. Angel proposed a theory suggesting that anemias are an adaptive response to malaria. There is a strong correlation in space and time with the distribution of anemias, malaria and porotic hyperostosis. Recent studies support the protective mechanism of thalassemia from severe and fatal malaria[18].

Epidemiologic data support the high prevalence of chronic hemolytic anemias in the current Cambodian population[19]. The Cardamoms is a well-known malaria infested region. The combination of these radiological and epidemiological data strongly suggests the diagnosis of thalassemia in two 500-year-old skulls. To the best of our knowledge, there is to date no previous report on the radiological patterns of thalassemia in ancient skeletal remains in Cambodia.

Conclusion

When cribra orbitalia is present in ancient skulls, X-rays and CT are useful to confirm bone changes especially when they are characteristic such as in the presence of "hair-on-end" pattern or coarsening of the trabeculae in the diploic space. However it is important to state that radiological findings lack specificity. The definite diagnosis of the hereditary anemias in ancient bone requires a correlation of radiological, anthropological, paleopathological and biological data such as DNA analysis.

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[15] Stuart-MacAdam 1992.

[16] Chou, Yen, Liang, Jong 2006.

[17] Angel 1966.

[18] Vento, Cainelli, Cesario 2006.

[19] Fucharoen and Winichagoon 1992.

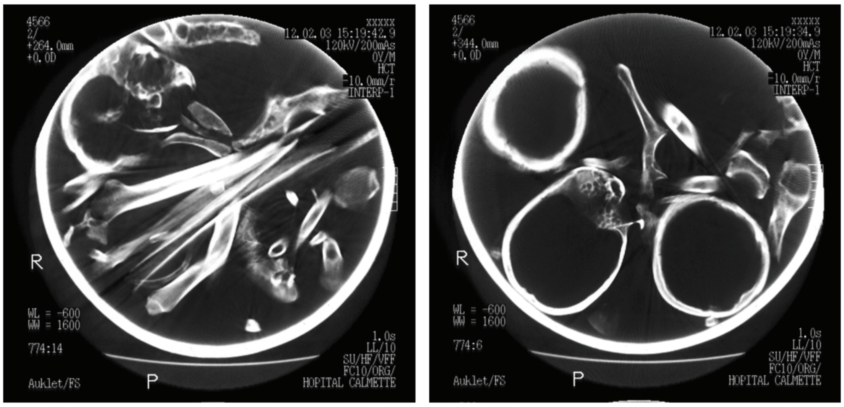
Caves in Cardamom Mountains

Burial jars in caves

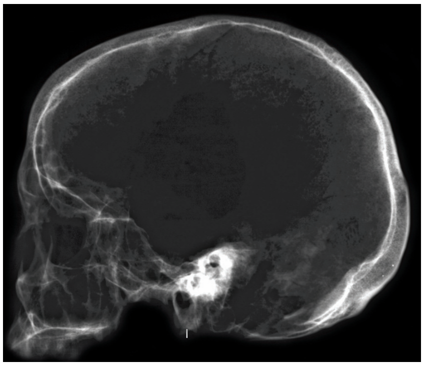
Skull and bones in a jar

Skull no1. Thickening of the skull

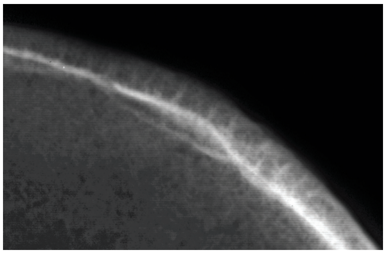
Skull 1. Cribra orbitalia



CT of skeletons-in-jar.



Skull no 1: Hair-on-end pattern.



Hair-on-end "blown-up" appearance.

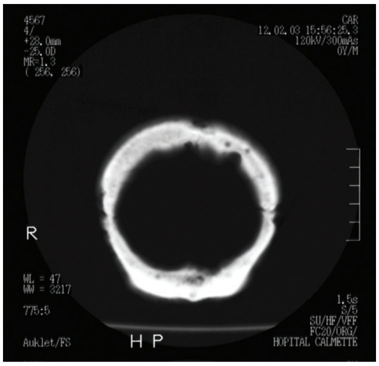
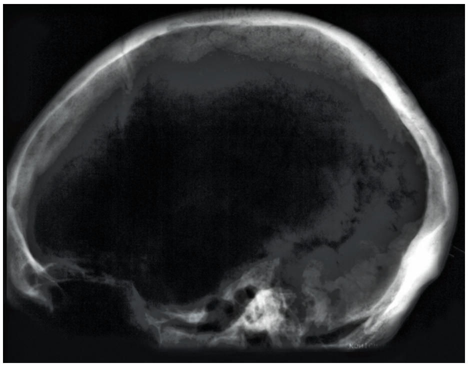
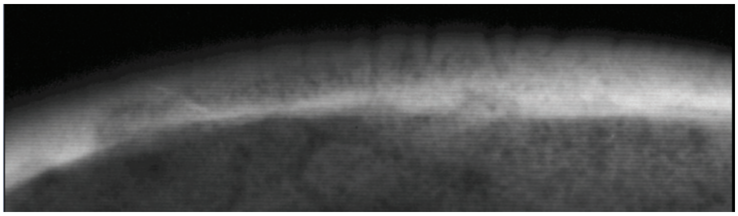


Fig 4C. CT of skull no 1



Skull no 2: Hair-on-end pattern



Hair-on-end "blown-up" appearance

References Cited

Agarwal, K.N., Dhar, N., Shah, M.M., Bhardwaj, O.P., "Roentgenologic changes in iron deficiency anemia", *American Journal of Roentgenology*, 1970, 110(3): 635-637.

Angel, J.L., " Porotic hyperostosis, anemias, malarias and marshes in the prehistoric Eastern Mediterranean", *Science*, 1966 August, 153(737): 760-763.

Aksoy, M., Camli, N., Erdem, S., "Roentgenographic bone changes in chronic iron deficiency anemia", *Blood* 1966, 27(5): 677-686.

Auferheide, A.C. and Rodriguez-Martin, C., "Hematological Disorders", *in*: *The Cambridge Encyclopedia of Human Paleopathology*. 1st ed. Cambridge, United Kingdom: Cambridge University Press, 1998: 345-350.

Chou, H.F., Yen, C.M., Liang, W.C., Jong, Y.J., "Diphyllobothriasis Latum: the first child case report in Taiwan", *The Kaohsiung Journal of Medical Sciences*, 2006 July, 22 (7): 346-350.

Daly, D., "Hair-on-end", *Pattern in the skull*. Seminars in Roentgenology 1987 July, ;22(3): 144-145.

Exner, S., Boqusch, G., Sokiranski, R., "Cribra orbitalia visualized in computed tomography", *Annals of Anatomy,* 2004 April, 186(2): 169-172.

Fucharoen, S. and Winichagoon, P. "Thalassemia in Southeast Asia: problems and strategy for prevention and control", *The Southeast Asian Journal of Tropical Medicine and Public Health*, 1992 December, 23(4): 647-655.

Hart, G.D., "Anemia in ancient times", *Blood Cells*, 1981, 7(3): 485-493.

Logothetis, J., Economidou, J., Constantoulakis, M., Augoustaki, O., Swenson, R.B., Bilek, M., "Cephalofacial deformities in thalassemia major (Cooley's Anemia)", *American Journal of Diseases of Children*, 1971 April, 121(4): 300-306.

Martin, M.A. *Les Khmer Daeum: Khmer de l'Origine. Société Montagnarde et Exploitation de la Forêt,* *de l'Écologie à l'Histoire*, Presses de l'École Française d'Extrême Orient, 1997.

Mosely, J.E., "Skeletal changes in the Anemias", *Sem Roentgenol*, 1974 July, 9(3):169-184.

Ponec, D.J., and Resnick, D., "On the etiology and pathogenesis of porotic hyperostosis of the skull", *Investigative Radiology*, 1984 Jul-Aug, 19(4): 313-317.

Reimann, F., Kayhan, V., Talati, U., Gokmen, "X-ray and clinical study of the nose, sinuses and maxilla in patients with severe iron deficiency diseases", *Laryngologie Rhinologie Otologie*, 1975 November, 54(11): 880-890.

Sebes, J.I., Diggs, L.W., "Radiographic changes of the skull in sickle cell anemia", *American Journal of Roentgenology*, 1979 March, 132(3): 373-377.

Steinbock, R.T., "Hematologic Disorders- the Anemias", *in*: *Paleopathological diagnosis and interpretation: Bone diseases in ancient human populations*, 1st ed. Springfield, Illinois: Charles C Thomas, 1976: 213-248.

Stuart-MacAdam, P., "A radiographic study of porotic hyperostosis", *American Journal of Physical Anthropology,* 1987 December, 74(4): 511-520.

Stuart-MacAdam, P., "Porotic hyperostosis a new perspective", American Journal of Physical Anthropology 1992 January, 87(1): 39-47.

Vento, S., Cainelli, F., Cesario, F., "Infections and thalassaemia", *The Lancet Infectious Diseases*, 2006 April, 6(4): 226-233.