

CASE REPORT

Tumoral Calcinosis in Infancy

A light and electron microscopic study with X-ray microanalysis

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The most frequent form of idiopathic calcinosis is tumoral calcinosis (TC) which rarely occurs at young ages. We describe here a TC case of a young boy with its light microscopy completed with electron microscopic examinations. X-ray microanalysis

revealed in the intracellular crystals CaCl_2 besides the previously described hydroxyapatite. The significance of this finding is unknown at the moment. (Pathology Oncology Research Vol 1, No1, 80-84, 1995)

Key words: tumoral calcinosis, infancy, microanalysis

Introduction

Calcification of soft tissues may be metastatic, dystrophic or idiopathic. Metastatic calcification is due to abnormal metabolism of calcium and/or phosphorus as in primary or secondary hyperparathyroidism, vitamin D toxicity, excessive alkali intake, carcinoma, multiple myeloma or other conditions.^{1,2,3} Dystrophic calcification occurs in tissue previously injured by trauma, ischemia or inflammation.¹ Idiopathic calcification most frequently appears as tumoral calcinosis (TC).⁴

Tumoral calcinosis – first described in the last century by French authors^{5,6} – is considered to be a familial disease represented by the appearance of calcified masses usually in the close vicinity of joints. Many cases of TC were previously described as lipocalcinogranulomatosis, calcinosis cutis circumscripta or generalized calcinosis. In TC the serum calcium level is normal, however, the phosphorus level may be variably altered.^{5,7,8} Interestingly, TC is more frequent in the first two decades of life but only a few cases have been reported under the age of two years.⁹ The light microscopic appearance of the lesion is characteristic: calcified material is surrounded by macrophages, histiocytes and multinucleated giant cells in soft tissue, in which the calcific debris contains hydroxyapatite.¹⁰

We describe here a case of TC in an unusually young North-African boy and provide a light- and electron microscopic analysis with a brief review of the relevant literature.

Materials and Methods

Light microscopy

Tissue samples were fixed overnight in phosphate buffered 4% formaldehyde, dehydrated and embedded in paraffin. Sections were stained with hematoxylin-eosin (H&E) or the periodic acid-Schiff reaction (PAS).

Electron microscopy

Formaldehyde fixed tissue samples were postfixed with 2% phosphate-buffered glutaraldehyde for 2 hrs, washed and postfixed in phosphate-buffered OsO_4 for 1 hr, dehydrated and embedded in Epon. Ultrathin sections were cut with a diamond knife and the sections were contrasted using uranyl acetate and lead nitrate. Photographs were taken on a Phillips CM10 electron microscope at an accelerating voltage of 80 kV.

X-ray microanalysis

Semithin sections of Epon embedded material were prepared using glass knives, mounted on copper-grids and studied without further contrasting. X-ray microana-

Received: Apr 10, 1995, accepted: May 15, 1995

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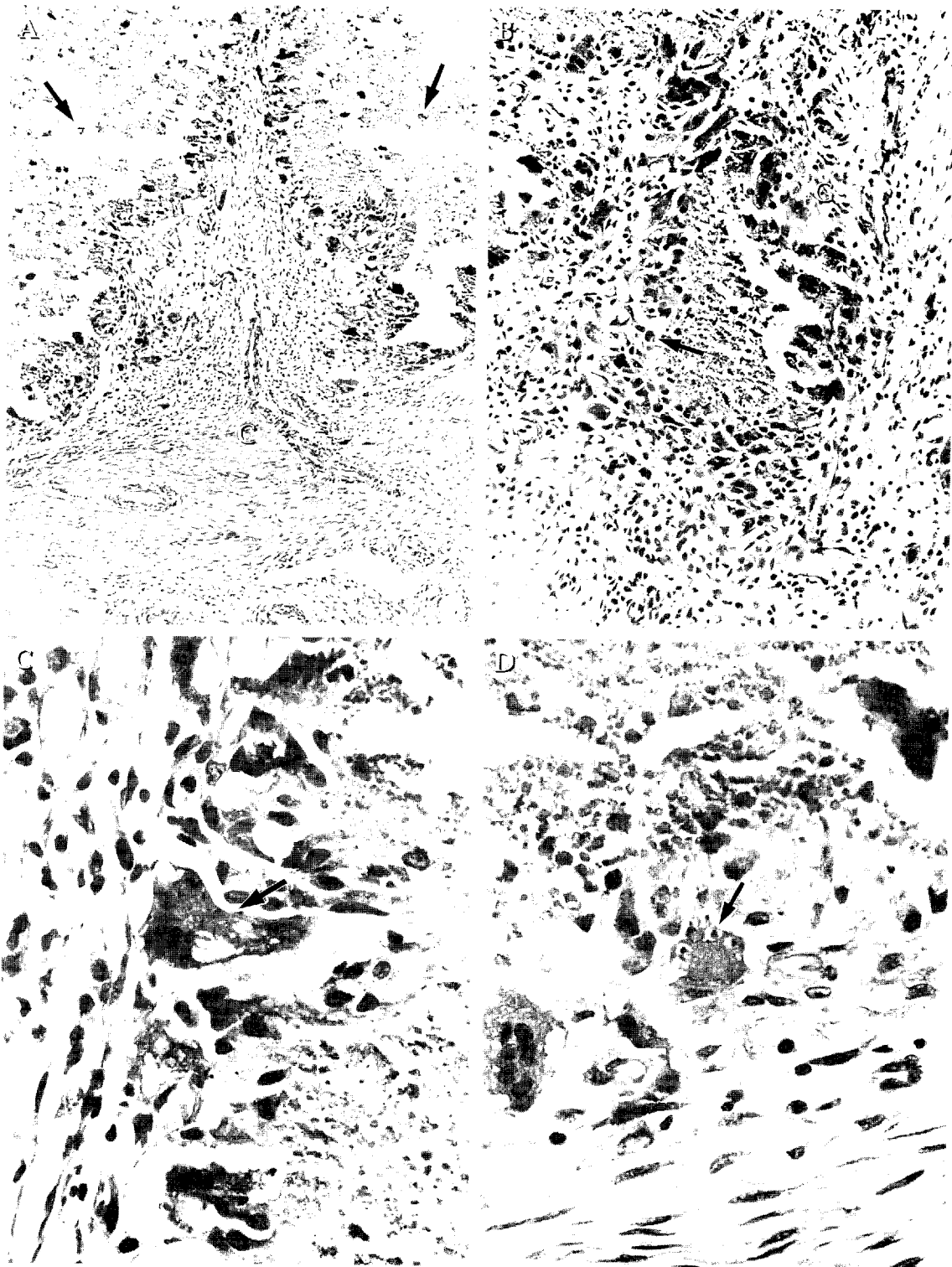


Figure 1. Light microscopy of tumoral calcinosis. A: Low power view of the lesion. Amorphous material (arrows) is surrounded by connective tissue (C). H&E stain - B: Amorphous material surrounded by multinucleated giant cells (G) and mononuclear cells (arrows). H&E stain. - C: High power view of multinucleated giant cell (arrow) with intracytoplasmic vacuole. H&E stain. - D: Multinucleated giant cell with intracytoplasmic PAS-positive granules (arrow).

lysis was performed in a JEM-100C electron microscope attached to an ORTEC-6230 energy-disperse X-ray microanalyser. Microanalysis was performed in STEM mode at 80 kV where specimen angle was 30° and collection time was 200 sec.

Case Report

A North-African black boy was hospitalised at the age of 15 months for a right supraclavicular mass which was tender and slowly growing. This mass was biopsied twice in North Africa and was diagnosed as a non-specific giant cell granuloma. The child was otherwise well and thriving. At the age of 30 months he was brought to the 2nd Pediatric University Hospital of Budapest. On admission a firm tender mass was found in the right supraclavicular fossa. MRI scan showed a 2-3 cm soft tissue mass at the apex of the thorax: the non-invasive mass was characterized by an inhomogenous structure and extensive calcification. The muscles and the major blood vessels were not involved. There were no dental or skeletal abnormalities. Serum calcium and phosphate levels were normal (2.4 and 1.6 mmol/l, respectively) as were the alkaline phosphatase (371 U/l) and serum creatinine (30 µmol/l) levels. Slight sideropenic anaemia (serum iron: 6.5 µmol/l, TIBC: 85.8 µmol/l) was present. There was no serologic evidence of past infections (CMV, EBV, HIV, toxoplasma, toxocara, echinococcus). No enlarged lymph nodes or calcifications were found on chest X-ray or with abdominal ultrasound examination. Considering the slow growth of the lesion and its close proximity to the big vessels and to the brachial plexus, the lesion was surgically removed. The resected tissue mass weighed 3.5 g, measured 2.5x3.3 cm and consisted of yellow granular material, soft tissue and unremarkable small lymph nodes.

Light microscopically the core of the supraclavicular mass consisted of amorphous basophilic material which was surrounded by a cellular zone of activated histiocytes and foreign body-giant cells (*Fig. 1 A-C*). These cells contained basophilic granules in their cytoplasm (*Fig. 1 D*) exhibiting PAS positivity. Outside the histiocyte layer there was a zone of proliferating capillaries and fibroblasts and the entire lesion was enclosed by a fibrous capsule. Ziehl-Neelsen and Gram stains were negative and no fungi were seen with the PAS reaction. The lymph nodes showed reactive changes with prominent sinusoidal histiocytes and endothelial cells.

Electron microscopy revealed histiocytes characterized by an extended cytoplasmic rim around their nucleus, well developed membranous organelles and electron dense cytoplasmic granules ranging in size between 10-200 nm. Large lipid droplets could also be recognized in the cytoplasm of these cells. Occasionally histiocytes contained large intracytoplasmic deposits of amorphous crystalline osmiophilic material (size of a few microns) (*Fig. 2 A*). X-ray micro-

analysis of the intracellular amorphous material identified three elements: Ca, P and trace amount of Cl; the ratio of Ca to P was 1.48 (*Fig. 2 B*). Chlorine was present in the intracellular inclusions in considerable amounts compared to the signal obtained from the material of the grid itself (Cu). In the extracellular mass (*Fig. 3 A*) only two elements were identified: Ca and P in a ratio of 1.38 (*Fig. 3 B*).

Discussion

Tumoral calcinosis is one of the most common benign tumors in Africa.¹¹ The published Anglo-American cases also were mostly black patients.^{12,13,14} Our patient has likewise been black and from Africa (Libya) – no family history was available. The disorder is characterized by usually single tumor-like deposits of calcium in the absence of widespread visceral or vascular calcification. The patients have normal serum Ca levels whereas in some cases the serum P level is elevated. Analysis of family histories indicates that TC is an autosomal recessively transmitted disorder in which family members without other manifestations of TC may show characteristic dental abnormalities.¹⁵ The age of onset of clinical signs is variable; TC has been described in patients of all ages but is most frequently seen in the second and third decades of life. Exceptionally it may be evident as early as the first month of life.⁹ In our case, too, there was an early onset of signs. Evidence of a hypothetic metabolic disorder may be present soon after birth. Uncapsulated calcified masses usually appear in close vicinity of joints. Other abnormalities such as hyperostosis or calcifications in mucous membranes, skin or skeletal muscle may also complicate the picture,¹⁵ especially in case of hyperphosphatemia. Metabolic studies reveal in almost half of the TC cases a detectable alteration in Ca, P and/or vitamin D metabolism in the epithelium of the renal tubules.^{16,17,18}

Morphologic studies of TC indicate a histiocytic reaction in the soft tissue around the calcified material. Ca can be found in the form of hydroxyapatite similar to that in bone where no other element can be detected in the crystal.^{10,19} The amorphous masses also contain collagen type-I, phospholipid and osteocalcin.¹⁹ Ultrastructural studies show that in TC calcification may start intracellularly in the endoplasmic reticulum of activated histiocytes where some protein material also could be detected¹⁹ as indicated by PAS positivity. In our case the intracellular amorphous material contained Ca and P in a ratio of 1.48 (similar to that of previous reports) as well as a significant amount of Cl. This finding indicates that beside hydroxyapatite, CaCl₂ may also be present, at least intracellularly. Interestingly, in our case Cl was not detectable in the extracellular hydroxyapatite crystals though the significance of this observation remains unknown. The differential diagnosis of TC includes tumoral lesions such as calcific bursitis, old fat necrosis or necrotizing granuloma with calcification.

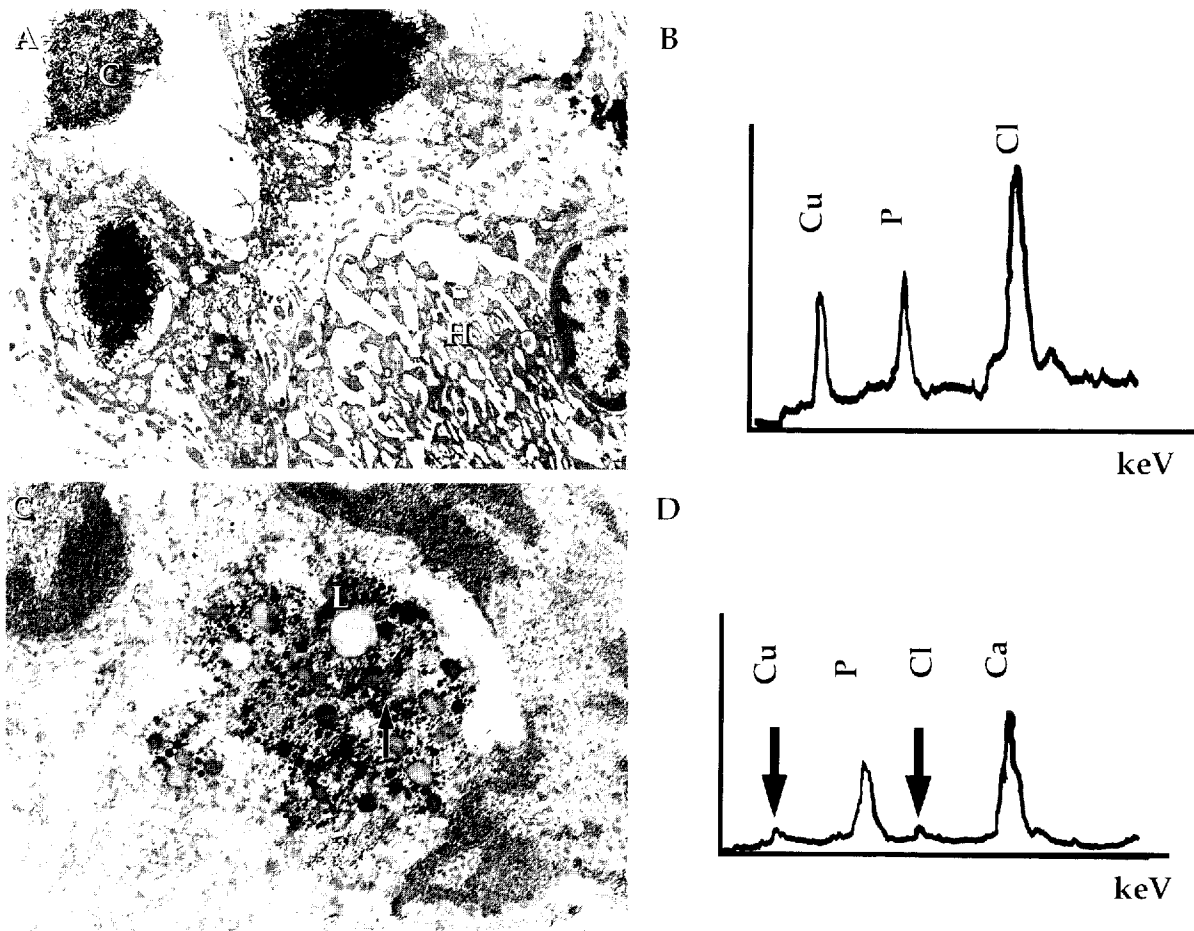


Figure 2. Electron microscopy of tumoral calcinosis. A: TEM view of extracellular electron dense crystalloid material (C) surrounded by an activated histiocyte (H), x 10,000 – B: X-ray microanalysis of the extracellular crystalloid material in STEM mode. Note the presence of La of Cu (copper grid), Ka of P and Ca. U=80 kV, $\alpha=30^\circ$, T=200 sec. – C: TEM view of a histiocyte in tumoral calcinosis. The cytoplasm contains lipid droplets (L) surrounded by electron dense granules (arrows), x 27,000 – D: X-ray microanalysis of the cytoplasmic electron dense material in STEM mode. Note the detection of La lane of Cu (copper grid) and Ka lanes of P, Cl and Ca. U=80 kV, $\alpha=30^\circ$, T=200 sec.

extraskeletal chondroma/chondrosarcoma/osteosarcoma, granular cell tumors and myositis ossificans. Moreover, metabolic disorders such as renal failure, excessive milk ingestion, vitamin D poisoning, hypoparathyroidism must also be considered.

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