Tumoral Calcinosis: Seasonal Biochemical Studies and Chemical Studies of Eyelid Lesion

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We recently described (Arch Ophthalmol 1988;106:725–6) the presence of unique calcific lesions in the eyelids of a young woman with a history of hyperphosphatemic tumoral calcinosis. Here we document that no immediate family members showed similar lesions and that none was hyperphosphatemic. Dental roentgenography revealed characteristic abnormalities in the patient that confirmed the clinical diagnosis of tumoral calcinosis. Seasonal biochemical studies demonstrated persistently increased concentrations of phosphorus and 1,25-dihydroxyvitamin D in her serum. A calcific eyelid excrescence removed from the patient, studied by x-ray diffraction, was found to consist of crystals of hydroxyapatite. Microprobe analysis indicated the major elements in the deposit to be Ca, P, S, and Cl, just as in the periarticular deposits found in tumoral calcinosis. The Ca concentration in the patient's tear fluid, measured by atomic absorption spectrometry, was within the range found in tears of healthy volunteers. Phosphorus was undetectable (<30 μmol/L) in tears of the patient and the volunteers. These findings suggest that the eyelid lesions represent a new manifestation of the pathological process that produces the characteristic periarticular calcific masses of tumoral calcinosis.

Additional Keyphrases: hydroxyapatite • calcium • phosphorus • 1,25-dihydroxyvitamin D • tears • circannual rhythms

Tumoral calcinosis is a rare syndrome characterized by benign recurrent deposits of hydroxyapatite in periarticular soft tissue (1). Typically, the patients are normocalcemic (2), but marked hyperphosphatemia has been reported in some (3). Serum 1,25-dihydroxyvitamin D frequently is increased (4, 5). Lyles et al. (6) recently identified a pathognomonic dental abnormality in these patients, which appears to be extremely useful for identifying affected individuals in family studies.

We (7) recently found unique ocular calcifications in a patient with tumoral calcinosis. We describe here clinical and biochemical studies of this patient and her family as well as the chemical characteristics of a calcific eyelid lesion and tears from the patient.

Case Summary

A 17-year-old white woman with a long history of tumoral calcinosis developed mild bilateral ocular irritation. On examination, a row of unique calcific lesions, each ~0.5 mm in diameter, was found on the inner surface of each lower eyelid. The full ophthalmological details are reported elsewhere (7). Periarticular calcific masses with characteristic histological appearance had been excised previously on four occasions. Although no periarticular masses were present at the time of examination, dental radiograms (Figure 1) revealed the pathognomonic findings of tumoral calcinosis described by Lyles et al. (6). The patient's father, mother, and only sibling were asymptomatic, with no family history of lesions suggestive of tumoral calcinosis or ocular problems.

Results of biochemical studies of sera from the patient's brother and parents (Table 1) were within appropriate normal reference intervals except for increased concentrations of 1,25-dihydroxyvitamin D and parathyrin in the mother. Radiographs of the mother's upper and lower jaw did not show the changes of tumoral calcinosis, and the mother did not exhibit ocular changes like those in the patient.

Relevant laboratory test results for the patient's serum included increased phosphorus (1.8 mmol/L) and increased 1,25-dihydroxyvitamin D (87 ng/L, 209 pmol/L). Serum chemical tests for which results were within normal limits included those for calcium, albumin, magnesium, creatinine, immunoreactive parathyrin, and 25-hydroxyvitamin D. Findings made during a year's observation of this patient were similar, revealing little seasonal variation (Figure 2).

Material and Methods

Tear samples were collected from the patient and from seven healthy age- and race-matched volunteers of both sexes, who had no history of eye disease or systemic illness. All the tear samples were collected in the morning, with no local anaesthetic, to avoid diurnal variations among the different specimens (8). A 25-μL fine-gauge glass micropipette was inserted into the lower conjunctival cul-de-sac, and the tear film was aspirated and transferred to a polyethylene microcentrifuge tube. The microcentrifuge tubes were centrifuged immediately for 3 min at 3000 × g to remove bacteria and debris, then stored at -20°C until analysis.
pete was positioned in the medial canthal lacrimal lake, while the lower lid was pulled down slightly. Care was taken not to induce reflex tearing. Tears, collected from both eyes and pooled to obtain the final sample, were analyzed for calcium by atomic absorption spectroscopy as described by Uttila et al. (9). We used a modification (aca; DuPont Co., Wilmington, DE 1988) of the phosphorus method of Daly and Ertlingshausen (10) for analysis of tear fluid. Tear pH was estimated with narrow-range pH paper.

A subconjunctival lid margin excrescence was unroofed and removed for X-ray diffraction studies (1). The sample was mounted on a fine glass capillary, and the diffraction pattern was obtained with a Debye–Scherrer camera with Ni-filtered Cu K-alpha radiation. The same sample was then mounted on a carbon planchette, sputter-coated with Pd, and subjected to microprobe analysis with an Amray 1000A EDAX scanning electron microscope. We recorded the patterns of the sample on the glass capillary, and of the glass capillary alone.

### Results

The calcium concentration in the patient's tear fluid, 0.39 mmol/L, was within the range found in the volunteers' tears (0.27–0.59 mmol/L; mean ± SD = 0.36 ± 0.11 mmol/L). Tear phosphorus concentration was below the lower limit of detection (~30 µmol/L) in all samples, whether from volunteers or patient. The tear pH was 7.0.

X-ray crystallography of the lid lesion revealed that it consisted of a poorly crystalline hydroxyapatite (Figure 3). Microprobe analysis indicated that the major elements in the deposit were Ca, P, S, and Cl (Figure 4).

### Table 1. Serum Biochemical Studies in Patient and Family Members

<table>
<thead>
<tr>
<th>Component</th>
<th>Reference</th>
<th>Interval</th>
<th>Mean ± SD</th>
<th>Median</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calcium</td>
<td>mmol/L</td>
<td>2.1–2.6</td>
<td>0.8–1.45</td>
<td>1.28</td>
</tr>
<tr>
<td>Phosphorus</td>
<td>mmol/L</td>
<td>1.84</td>
<td>150–600</td>
<td>37–69</td>
</tr>
<tr>
<td>25-OH-D</td>
<td>ng/mL</td>
<td>0.3–1.3</td>
<td>10000</td>
<td>4400</td>
</tr>
<tr>
<td>25(OH)2D</td>
<td>ng/mL</td>
<td>0.2–0.59</td>
<td>59–74</td>
<td>0.03–1.1</td>
</tr>
</tbody>
</table>

The values are means ± SEM of *n* = 4, except for the Parental samples, which are *n* = 7.

Fig. 2. Seasonal biochemical studies in patient with tumoral calcinosis: serum concentrations of phosphorus, calcium, albumin, magnesium, 25-hydroxyvitamin D(25-OH-D), 1,25-dihydroxyvitamin D[1,25(OH)2D], and parathyroid (PTH) is shown. Shaded areas denote normal reference intervals.

Fig. 3. Wide angle X-ray diffraction pattern (Cu K-alpha radiation) of deposit from eye (top) and hydroxyapatite standard (bottom). Central broad band in top pattern is due to presence of organic material in the deposit, all other lines are due to poorly crystalline hydroxyapatite.

Fig. 4. Elemental analysis of ocular deposit (dots) and background (white line) shows the presence of Ca, Cl, S, and P in the deposit. Pd and Si reflect Pd-coating of specimen and the use of glass capillary, respectively.
Discussion

This patient had a characteristic history of hyperphosphatemic tumoral calcinosis with increased serum 1,25-dihydroxyvitamin D. The periartrial locations of the previously removed lesions were typical of this disorder, as were the normal serum concentrations of calcium and parathyрин. The dental findings shown in Figure 1 confirmed the diagnosis of tumoral calcinosis. Seasonal studies in this disorder have not been reported previously. The unusual finding in this patient was the presence of ocular involvement.

Several lines of evidence suggest that the lid lesions in this patient represent a new manifestation of tumoral calcinosis. Similar lesions have been reported in no other disorders or in healthy individuals (7). Members of the patient’s immediate family had no such lesions. Secondly, the lesions consisted of hydroxyapatite crystals, as do the periartrial lesions of the disorder (1). Finally, the elemental composition of the lid lesions, including the presence of sulfur, is identical to that found in the characteristic periartricular calcifications of this disorder (1).

What factors promote deposition of hydroxyapatite in and around the eye is not known. Factors associated with ectopic hydroxyapatite formation include increases in local calcium and phosphate concentration, formation or exposure of hydroxyapatite nucleators, and removal or modification of inhibitors of hydroxyapatite formation (11). The hyperphosphatemic state of the present patient may have contributed to the formation of hydroxyapatite in and around her eye. Hyperphosphatemia is a common feature of tumoral calcinosis. The concentrations of calcium (0.38 mmol/L) and phosphorus (<0.03 mmol/L) in tears of this patient suggest that high tear concentrations of calcium and phosphate were not responsible for the deposits. Similar to reported calcific deposit in Bruch’s membrane of the eye (12), the deposit seen in this patient contained sulfur, presumably from glycosaminoglycans or proteoglycans. This suggests that alterations in proteoglycan content or composition, as discussed elsewhere (13, 14), may have contributed to the proliferation of hydroxyapatite seen in this patient. Alternatively or additionally, the increased concentrations of phosphorus and 1,25-dihydroxyvitamin D in serum may be critical.

The mechanism by which calcifications occur specifically in the lid margin is open to conjecture. The location of the deposits, close to the point where the lid comes in contact with both the tear film and the atmosphere, might suggest that a localized alteration in pH caused by loss of tear CO₂ promoted deposition of hydroxyapatite in this site, a mechanism similar to that which has been proposed for the ocular calcification of band keratopathy (15). In this situation we would have expected increases in either the calcium or the phosphorus concentration of the tear film, but we found evidence of neither.

In summary, the chemical properties described here suggest that the eyelid lesions in our patient represent a manifestation of tumoral calcinosis. The family studies and biochemical studies and the analyses of tear fluid are consistent with this conclusion. We suggest that the calcific excrescences on the lower lid, like the dental findings first described by Lyles et al. (6), represent a potential new tool in family studies of tumoral calcinosis. Further studies are required in larger kindreds to determine the prevalence of the lid lesions in individuals who are affected with tumoral calcinosis.

References