References

1. Srivastava S, Jaiswal R, Agarwal A, Singh PK, Singh SN. Cytodiagnosis of adenoid cystic carcinoma of the parotid metastatic to kidney and lung. J Cytol 2007;24:201-2.
2. Florentine BD, Fink T, Avidan S, Braslavasky D, Raza A, Cobb CJ. Extra-salivary gland presentations of adenoid cystic carcinoma: A report of three cases. Diagn Cytopathol 2006;34:491-4.
3. Nayak SP, Dekate RT, Kotwal MN, Bobhate SK. Cytodiagnosis of adenoid cystic carcinoma of breast: A case report. J Cytol 2006;23: 149-50.
4. Jones AS, Hamilton JW, Rowley H, Husband D, Helliwell TR. Adenoid cystic carcinoma of the head and neck. Clin Otolaryngol Allied Sci 1997;22:434-43.

Richa Pandey, PK Singh, Lubna Khan
Department of Pathology, GSVM Medical College, Kanpur, Uttar Pradesh, India.

For correspondence:
Dr. Richa Pandey, U-11/1, Badshah Nagar estate Colony, Mahanagar, Lucknow, Uttar Pradesh, India.
E-mail: drrichapandey@gmail.com

DOI: 10.4103/0970-9371.50805

Cytodiagnosis of tumoral calcinosis

Sir,

Tumoral calcinosis is a rare clinico-pathological entity that is distinct from other calcific lesions such as the milk-alkali syndrome, hypervitaminosis-D myositis ossificans, and hyperparathyroidism associated with chronic renal failure.

A 45 year-old male presented with a firm, irregular, painless, soft tissue mass of six months' duration, measuring 3 × 2 cm around the elbow joint, with normal overlying skin. The lesion remained localised without the involvement of any adjoining structures; there was no history of trauma. General and systemic examination results were normal. A clinical diagnosis of a parasitic lesion was considered along with the possibilities of a skin adenexal tumor, schwannoma, neurofibroma, and lipoma. Blood eosinophil and absolute eosinophil counts (AEC), and serum calcium and phosphorus levels were normal. Radiograph showed features of an osseous, lytic lesion. Fine needle aspiration cytology (FNAC) was performed using a 22 gauge needle and a 10 mL disposable syringe to aspirate grey-white granular material for the preparation of smears. The smears were fixed in 95% alcohol and stained with hematoxylin and eosin (H and E), whereas the air-dried smears were stained with May-Grünwald-Giemsa (MGG) stain. Microscopy showed clumps of intense, basophilic, amorphous calcium deposits [Figure 1] along with few ill-defined granulomas in the background. A diagnosis of tumoral calcinosis was rendered and surgical excision was done. On gross examination, the lesion was found to be firm, irregular, and 3 × 2 cm in size. The cut section was gritty and showed whitish, chalky areas of calcification. Multiple sections were taken and stained with H and E and von Kossa stain. Microscopy showed the accumulation of calcium deposits without any necrosis or collection of eosinophils or lymphocytes [Figure 2] and no evidence of dead or living parasites. The sections stained with von Kossa stain were strongly positive [Figure 2 inset]. Histological staging was done as per the criteria suggested

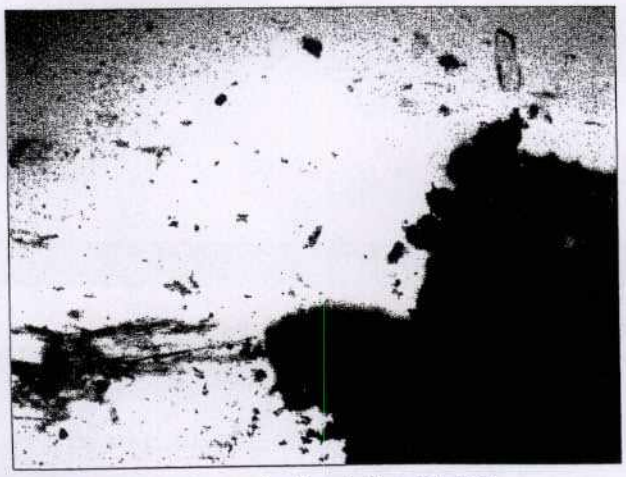

Figure 1: FNA smear of tumoral calcinosis (H and E, x200)

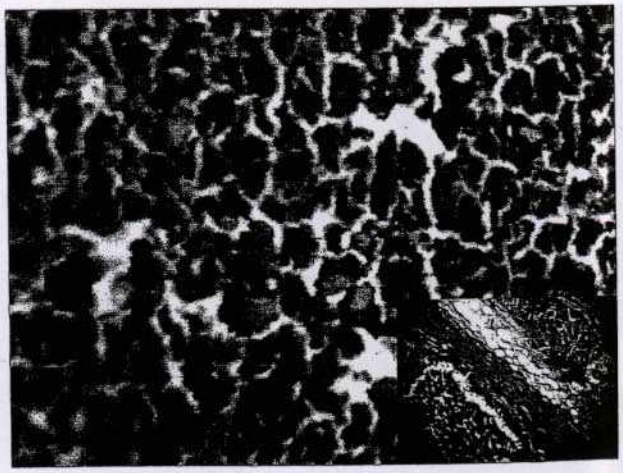

Figure 2: Microphotograph of tumoral calcinosis(H and E, x200), inset shows Von Kossa stain

by Thomson *et al.*[1] and Veress *et al.*[2] and a final diagnosis of stage I tumoral calcinosis was made.

Tumoral calcinosis is a variant of idiopathic calcinosis cutis and consists of numerous, large, painless, calcified subcutaneous masses in juxta-articular sites, particularly around the hip, shoulders, and elbows. Occasional cases have been reported in the distal femur and neck and multiple, concurrent, or asynchronous lesions have been reported.[3] There is no sex preponderance, but it shows a familial tendency.

Shivkumar *et al.*[4] studied the cytological features of idiopathic scrotal calcinosis and noted the presence of intense, basophilic, amorphous, granular deposits surrounded by lymphocytes, histiocytes, and foreign body giant cells without any evidence of epithelial cells in the smears. Recent studies have shown that tumoral calcinosis is a form of dystrophic calcification related to mechanical trauma or injury.[5] This theory is favored by the fact that the distribution of the lesions occurs predominantly in pressure points, and in the rural population whose poor socio-economic conditions mean that they sleep on the ground or on a wooden board or a hard floor, which in turn, damages the collagen, possibly by pressure ischemia and ultimately, leads to calcification and the formation of tumoral calcinosis.[5]

A thorough knowledge of this entity will reflect the true incidence of this lesion and avoid several diagnostic pitfalls.

References

1. Thomson JF, Tanner FJ. Tumoral calcinosis. J Bone Joint Surg Am 1949;31:132-40.
2. Veress B, Malik MO, Hassan AM. Tumoral lipocalcinosis: A clinico-pathological study of 20 cases. J Pathol 1976;119:113-7.
3. Inclan A. Tumoral calcinosis. JAMA 1943;121:490-5.
4. Shivkumar VB, Gangane N, Kishore S, Sharma S. Cytological features of idiopathic scrotal calcinosis. Acta Cytol 2003;47:110-1.
5. Pinto RG, Baretto G, Rodrigues J. FNAC of calcinosis cutis. J Cytol 2005;22:1997.

Subhashish Das, Kalyani R, Harendra Kumar ML

Department of Pathology,

Sri Devaraj Urs Medical College, Tamaka, Kolar, India

For correspondence:

Dr. Subhashish Das,

C/O Dr. Kalyani. R., H. No.127/13,

"Sri Ganesh", 4th main, 4th cross,

P.C. Extension, Kolar - 563 101, Karnataka, India.

E-mail: daspathology@gmail.com

DOI: 10.4103/0970-9371.50806

Table 4 Anticoagulant induced other artefacts

Time	Smudge cells		Swollen WBC'S		Crenated RBC'S		Abnormal staining of WBC'S	
	EDTA	SOD.CIT	EDTA	SOD.CIT	EDTA	SOD. CIT	EDTA	SOD.CIT
0 HR				11 (22%)		10 (20%)		06 (12%)
1 HR	05 (10%)	27 (54%)		29 (58%)		14 (28%)		07 (14%)
2 HR	26 (52%)	21 (42%)	19 (38%)	10 (20%)	23 (46%)	18 (36%)	04 (08%)	30 (60%)
3 HR	16 (32%)	02 (04%)	31 (62%)		13 (26%)	08 (16%)	40 (80%)	07 (14%)
4 HR	03 (06%)	M	M	M	14 (28%)		06 (12%)	
5 HR	M	M	M	M	M	M	M	M
6 HR	M	M	M	M	M	M	M	M

Abbreviations: HR-hour; EDTA-ethylene diaminetetraacetic acid; Sod.Cit-sodium citrate; M-marked changes

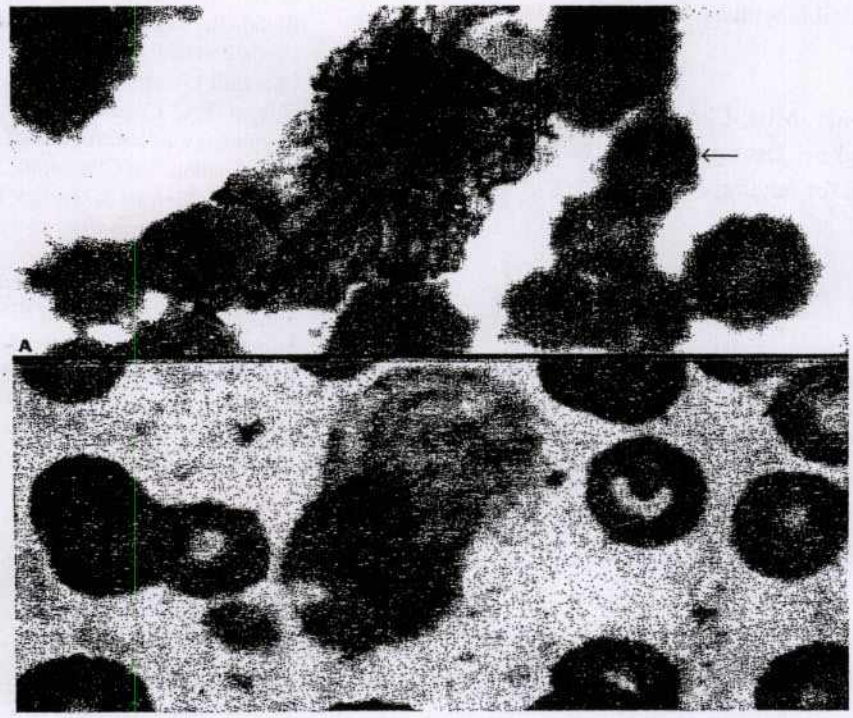

Fig. 6 Photomicrograph showing other artefacts. **(6a)** Smudge cell with crenated RBC's (Thin arrow). **(6b)** Swollen WBC. (Leishman, X 1000)

cytoplasmic blebs and finally cytoplasmic rupture. These changes began as early as '0' hr with sodium citrate with completely altered morphology at the end of 6 hrs, but delayed up to '2 hrs' with EDTA blood. The cytoplasmic vacuoles are clear and discrete with uneven distribution of cytoplasmic granules along with irregular cell membrane which was consistent with the findings of Vajpayee [5], Eastham [6], Lewis [7] and Gosett [8].

Smudge cells, increase in size of the WBC's, crenated RBC's and abnormal staining characters as described by Raphael [3] and Vajpayee [4] were also observed in our study which began as early as '1 hr' with citrate but delayed up to '3–4 hrs' with EDTA blood.

Platelets: Swelling of platelets occurred as early as '2 hrs' with EDTA and '1 hr' with citrate. Spiny projections were also observed. Zeigler observed that if films are made at 3 hrs after blood drawing, the fraction of large platelets are increased.[9]

Finally, EDTA induced pseudo-agglutination of platelets were initiated at '3 hrs' and marked at '6 hrs'. No significant platelet aggregation was observed with citrate blood.

This finding of our study was comparable to that of Shimasaki et al [10], Lippi et al.[11] At '6hrs' all these changes are marked, but more severe with citrate than EDTA [7].

Hence, preparation of satisfactory blood smears requires careful attention to preparation of blood smear with proper

use of appropriate anticoagulant and staining techniques with sound knowledge of morphologic appearances of normal and pathologic cell types.

Conclusions

EDTA has been recommended as the anticoagulant of choice for peripheral blood smear as it allows the best preservation of cellular components and morphology of blood cells. Citrate should be avoided as it may result in increase cell lysis and altered morphology. It is advisable to make smears immediately with the anticoagulated blood. A delay up to 1hr is permissible with EDTA blood but not beyond.

Acknowledgement Miss. Christina (Lab Technician) for technical help and Sri Devraj Academy of Higher Education and Research for funding.

References

1. Mills JN (1998) Interpreting blood smears (or what blood smears are trying to tell you!). Aust Vet J 596:1–8
2. Perkin SL (2004) Examination of Blood and Bone Marrow. In: Green JP, Foerster J, Lukens JN, Rodgers GM, Paraskevas F, Glader B, Eds. Wintrobe's Clinical Haematology. (11th Edition). Philadelphia: Lippincott Williams & Wilkins, 4–12
3. Raphael SS (1983) Principles of Haematology. In: Raphael SS, Eds. Lynch's Medical Laboratory Technology. (4th Edition). Philadelphia: Saunders, 126
4. Sacker LS (1975) Specimen Collection. In: Lewis SM, Coster JF (eds). Quality Control in Haematology. New York: Academic Press, 211
5. Vajpayee N, Graham SS, Bem S (2007) Basic Examination of Blood and Bone Marrow. In: Mc Pherson RA, Pincus MR, Eds. Henry's Clinical Diagnosis and Management By Laboratory Methods. (21st Edition). India: Saunders, 476
6. Eastham RD (1985) Peripheral White Blood Cells. In: Eastham RD, Eds. Clinical Haematology. (6th Edition). England: John Wright & Sons Ltd, 178
7. Lewis SM, Tatsumi N (2006) Collection and Handling of Blood. In: Lewis SM, Bain BJ, Bates I, Eds. Dacie And Lewis Practical Haematology. (10th Edition). Philadelphia: Churchill Livingstone, 8
8. Gossett KA, Carakostas MC (1984) Effect of EDTA on morphology of neutrophils of healthy dogs and dogs with inflammation. Vet Clin Pathol 3(2): 22–25
9. Zeigler Z, Murphy S, Gardner FH (1978) Microscopic platelet size and morphology in various hematologic disorders. Blood, 51:479–486
10. Shimasaki A, Kato T, Ozaki Y (1994) Studies of platelet aggregation in six cases of EDTA- dependent pseudothrombocytopenia. Rinsho. Ketsueki 35(6):529–534
11. Lippi U, Schinella M, Nicoli M, Modena N, Lippi G (1990) EDTA- induced platelet aggregation can be avoided by a new anticoagulant also suitable for automated complete blood count. Haematologica 75(1):38–41