Original Article

DOI: 10.21276/APALM.1466

Heterometaplastic Bone Formation in Nephrolithiasis: Critical Review of Pathology and Pathogenetic Mechanisms

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ABSTRACT

Background: We critically analyze the incidence, presentation and histopathologic findings of heterometaplastic bone formation (HBF) in nephrolithiasis in the kidneys of patients undergoing percutaneous nephrolithitomy for stone disease.

Methods: Percutaneous nephrolithitomy (PCNL) was performed on 932 patients from August 2009 to October 2016 by a single surgeon. In 43 cases, heterometaplastic bone formation was seen to originate from urothelium and encompassing the renal calculi. Clinical workup, radiographic imaging, treatment modalities and histopathologic features in these patients were evaluated.

Result: The patients' age ranged from 14 years to 65 years (median age 33.7 years). The male to female ratio was 4.3: 1.Heterometaplastic bone formation (HBF) encompassing the stone was identified in 69.76% in right kidney, 25.58% in left kidney and 4.65% in both kidneys. Radiographic appearance of eccentric density surrounding hypodense area was observed in 32 of 43 cases (74.41%). Histopathological evaluation showed trabecular bone with surface osteoblastic activity and intra trabecular bone marrow, haemopoietic cells and adipose tissue encompassing birefringent crystal deposits in 22 cases (51.16%). Trabecular bone in intimate proximity of woven bone and haemopoietic cell islands partially encompassing birefringent crystal deposits was observed in 17 cases (39.53%). Woven bone with mineral deposits and fibro collagenous proliferation was seen in 4 cases (9.30%).

Conclusion: Although reported infrequently, HBF in nephrolithiatic deposits has a high incidence in our patients. Pathogenetic mechanisms regarding transdifferentiating renal stem cells appears tenable in such a setup and is corroborated in our study.

Keywords: *Bone, Crystals, Heterometaplastic, Nephrolithiasis*

Introduction

Heterometaplastic bone formation in stone pathology has been rarely described in the kidney. The first recorded bone formation in the pedicle attachment of a renal calculus to the kidney pelvis has been recorded in 2 cases of nephrectomy. [1] Stuart and Krikorian reported occurrence of true bone within a renal calculus. [2] Cifuentes Delatte et al have reported osseous and cartilaginous metaplasia in 1.17% of 1624 urinary stones. [3] With the advent of PCNL the reported incidence has shown a rise upto 3.4%. [4] The typical radiographic and histopathologic features have been found fairly commonly in our practice of renal stone surgeries. We present 43 cases of heterometaplastic bone formation predominantly encompassing renal stones requiring modified surgical interventions in 932 PCNLs performed in 2117 patients treated for renal stone disease at our institution. This series highlights the peculiarities of heterometaplastic bone formation and the implications of unexplored pathogenesis about epithelial-mesenchymal trans-differentiation of urothelial stem cells towards osteogenic lineage.

Materials and Methods

2117 patients of nephrolithiasis required therapeutic surgical intervention of various types. Nephrolithotomy (PCNL) was performed in 932 patients at the institute of urology between August 2009 and October 2016. All the patients presenting with renal stones underwent preoperative clinical assessment, excretory urography, plain X ray (KUB) and CT scan. PCNL was performed under general anaesthesia with endotracheal tube. Retrograde pyelography was performed prior to PCNL access. Access was acquired under fluoroscopic guide using 17Fr or 26Fr nephroscope. Stones were fragmented with pneumatic lithoclast. After clearance and debulking, the pelvicalyceal system was inspected with a nephroscope. Whenever abnormal appearing hard pelvic tissue, bleeding stone, adherent stone was visualized, biopsy was taken and sent for histopathological examination.

Bony tissue when identified, the undecalcified tissue was processed for paraffin embedded section and stained by haematoxylin and eosin, trichrome stain and visualized by light and polarizing microscopy.

Decalcification was avoided for proper visualization and evaluation of heterometaplastic bone. 43cases revealing heterometaplastic bone with crystalloid deposits have been documented. The clinical, radiographic and operative records were reviewed for patient's age, sex, location of stones and the operative findings. These findings and histopathological details were evaluated.

Result

Heterometaplastic bone formation and crystal deposits were seen in 43out of 932 consecutive PCNL procedures (4.61%). These HBF with renal stones included one child (2.32%) and 42 adults (97.68%) with a mean age of 33.7 years(age range 14 to 65 years). The male to female ratio was 4.3:1.The duration of symptoms in these 43 patients ranged from 3 months to 42 months. Preoperative urine analysis showed Calcium Oxalate (CaOx) crystals in 27 out of 43 cases. Bacteriological culture identified urinary tract infection in 8 patients preoperatively. HBF was identified in 69.76% in right kidneys, 25.59%.in left kidneys and 4.65% in both kidneys. Preoperative nephroscopic appearance was that of a hard looking stone adherent to the pelvic mucosa. During pulverization it was found that the stone had an admixture of bony hard tissue. During removal, the white hard tissue was in continuation with the mucosal lining of the papilla/renal pelvis. The removal was accompanied with operative area bleeding which was duly controlled. Of the 43 cases, the HBF encompassing the stone was located near the renal papillae in the pelvi-calyx in 40 cases and 3 near the right PUJ .These stones were grey to dark brown in colour, 4mm to 25mm in size and weighing from340mg to 2100mg.

The X ray findings were available in 43 cases where typical findings were seen in 32 cases (74.41%), where plain films revealed radio-opaque eccentric halo with radiodensity showing connection with the urothelium. 11 cases (25.58%) revealed only fluffy radio-opaque densities. Stone analysis in the 43 cases showed predominantly CaOx in 35(81.39%) with additional urates in 14(32.55%), mixed struvite stones in 8(18.60%) cases. In all the surgical resections, the tissue was hard and brittle in consistency. .

Histopathologic evaluation showed 3 patterns-

Pattern I showed trabecular bone with surface osteoblastic activity and intra trabecular bone marrow, haemopoietic cells and adipose tissue completely encompassing birefringent crystal deposits in 22 cases (51.16%). (**Figure 1A, 1B, 1C and 1D).**

Pattern II showed trabecular bone in intimate proximity of woven bone and haemopoietic cell islands partially encompassing the birefringent crystal deposits was observed in 17 cases (39.53%) (**Figure 2A, 2B and 2C**)

Pattern III showed woven bone with mineral deposits and fibro collagenous proliferation was seen in 4 cases(9.30%) (**Figure 3A and 3B).**

In 39 specimens from the first and second group, bony areas were clearly seen in continuation with the urothelium. **(Figure 1B).** Chronic inflammatory changes, urothelial proliferation and subepithelial spindle cell proliferation was evident in 28 cases (71.79%).

Discussion

Metaplastic changes along urothelium denote a deranged epithelial response to injurious stimuli. These changes have been variously named as ectopic renal ossification, [3] bone formation in ureter, [5] ossification in the kidney stone, [1] pyelic osseous formation, [6] bone metaplasia, [7,8] extra osseous metaplasia [9] and extra osseous bone formation in renal pelvis . [4] The terminology of Heterometaplastic bone formation used in the present study denotes formation of tissue foreign to the part where it is formed. [10] These responses initially were hypothesized as Randall's plaque. [11] The sites of interstitial crystal deposition were shown to be near the tip of papillae. He conjectured CaOx stone formation at these sites which was confirmed by others [12-16] and recognized this discovery adequately to be an important step in our understanding of pathogenesis of HBF.

Over the last three decades, various hypotheses and observations have been put forward as 1) hypothesis of physico-chemical imbalance; 2) fixed particle theory; 3) role of defective renal tubular cells; & 4) discovery of crystal growth and aggregation inhibitors including macromolecules such as osteopontin, nephrocalcin, bikunin and BMP-2. [17-26]

Gambaro et al have been unable to combine these hypotheses with the hypothesis that Randall's plaque

Table 1: Showing Histopathological patterns in heterometaplastic bone formation of 43 cases.

Serial No.	Histopathological patterns	No.of cases	Percentage	Continuation with urothelium
1	Pattern I	22	51.16%	Present
2	Pattern II	17	39.53%	Present
3	Pattern III	4	9.30%	Absent

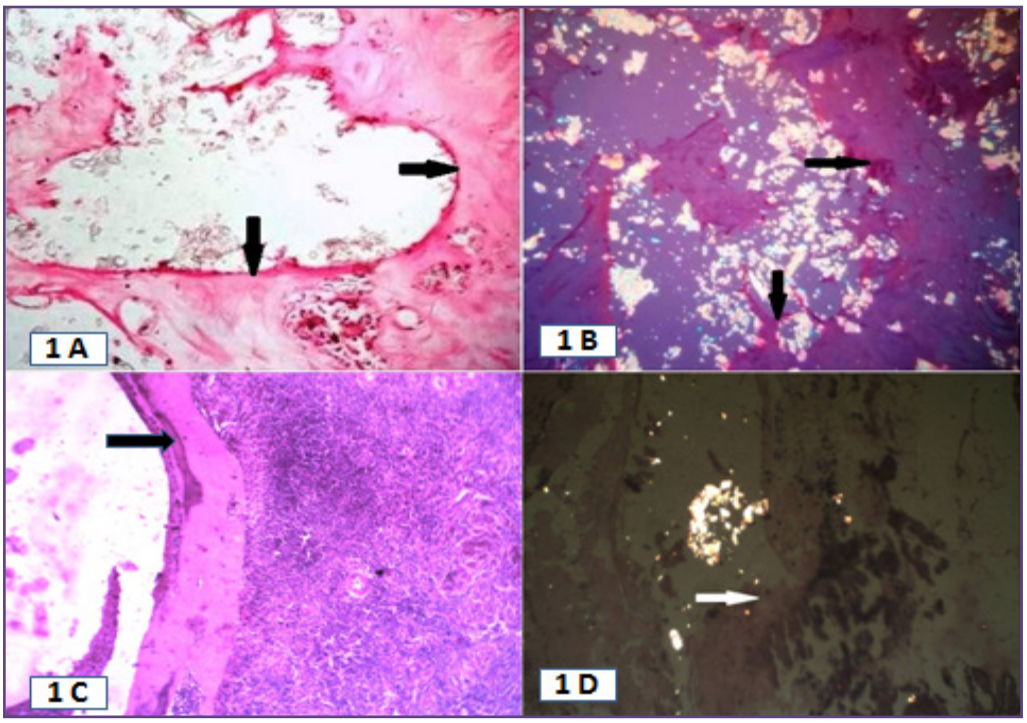

Fig. 1: Pattern I-Trabecular bone (arrow) completely encompassing birefringent crystal deposits , Fig. 1A (H & E X 400), Fig. 1B (Polarising microscopy X 400), bony areas (arrow) were clearly seen in continuation with the urothelium. Fig. 1C (H & E X 400) birefringent crystal deposits with trabecular bone (arrow) Fig. 1D (Polarising microscopy X 400).

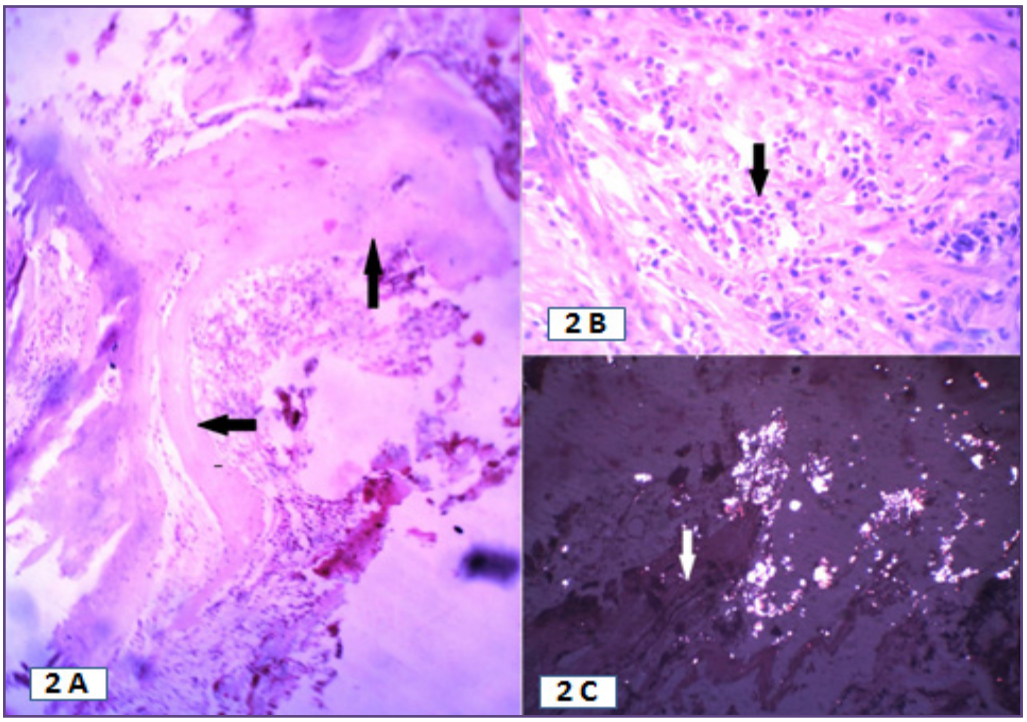

Fig. 2: Pattern II-Trabecular bone (arrow) partially encompassing birefringent crystal deposits Fig. 2A (H & E X 400), trabecular bone in intimate proximity of woven bone and haemopoietic cell islands (arrow) Fig. 2B (H & E X 400), trabecular bone (arrow) partially encompassing birefringent crystal deposits Fig. 2C (Polarising microscopy X 400).

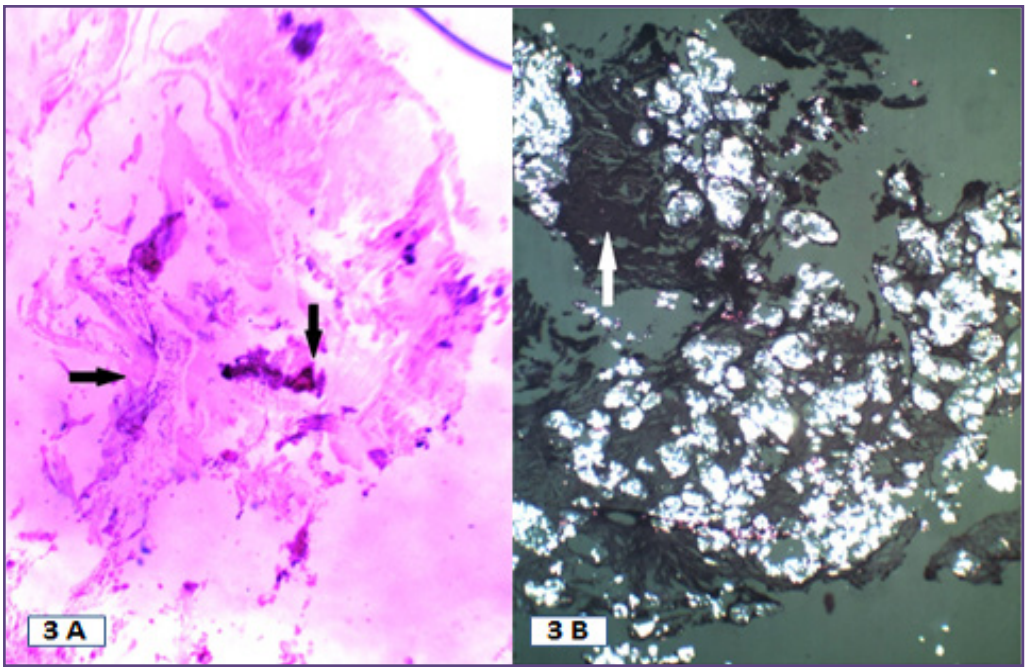

Fig. 3: Pattern III-Woven bone (arrow) with significant mineral deposits and fibro collagenous proliferation (arrow) Fig. 3A (H & E X 400), Fig. 3B (Polarising microscopy X 400).

allows CaOx stones to form and grow in the renal pelvis. [17] In vitro models, oxalates have been shown to trigger inflammatory, oxidative, chemotactic and fibrogenic loops. [18-20] Gambaro et al have hypothesized regarding condition which may trigger the trans differentiation of tubular cells the origin of which is mesodermal despite their epithelial appearance. [21] Thus the epithelial cells may be induced to undergo epithelial-mesenchymal differentiation under paraphysiological oxalate concentrations similar to idiopathic CaOx stone formers (ICSF). The renal interstitial myofibroblasts like the liver Ito cells are thought to be pericyte like cells. [22, 23] Notably such pericytes have the ability to undergo osteoblastic differentiation and mineralization. [24-25] Cultured artery smooth muscle cells, similar to multipotent interstitial cells in the kidney are also induced to become osteogenic by inflammatory stimuli, reactive oxygen species and hypoxia. [26]

Given its particular conditions of low oxygen tension, the papilla is a niche for stem cells, which have been shown to differentiate into myofibroblasts and cells expressing neuronal markers and to spontaneously form cellular spheres. These renal stem cells can migrate to other parts of the kidney and to the medullary tubular epithelia in particular. [27-28] Since stem cells recovered from other tissues can differentiate along the bone lineage, the third cell population potentially capable of mineralizing in the kidney is that of papillary stem cells.

Taking into consideration the peculiar physiological condition of the papillae, of low oxygen tension; and a sub-ischaemic environment, the pericyte like stem cells are sensitive even to mild toxic insults, or to high CaOx or phosphate concentrations and their propensity towards osteogenesis should explain such high occurrence of HBF in renal stones in our cases.

Huggins has confirmed the sequential steps of ectopic osteogenesis. [29] The appearance of small cysts to calcification, then to organized osseous tissue was confirmed in experiments on urothelial tissue. It was hypothesized that the primary osseous metaplasia has served as a focus for superimposed stone formation. This view was supported by Fernandez Conde et al ,who have suggested deposition of woven bone which later on was remodeled to form lamellar bone. [8] This ossification nidus perforating the urothelium comes in contact with urine. The direct and continuous action of urine induces bone formation. In our series, the HBF was found in renal papilla and in the renal pelvis. The complete encompassment of the stone by heterometaplastic bone suggests the involvement of multi-potent papillary stem cells. These stem cells convert to osteoblastic cells due to humoral inductions. Bone encompassing the kidney stones has been studied using undecalcified biopsied material. This approach has been found to conserve cellular and extracellular details and has been utilized in the present study. [8]

In a single case of extraosseous bone formation by Stuart et al and Plata, the entire stone was described as osseous tissue. [2, 9] In the present study we have seen the stone encompassed by heterometaplastic osseous tissue, crystals forming the nidus confirmed by polarizing microscopy. In the present study, sections from all stones revealed firm adhesions with urothelial mucosa by fibrous tissue or bone. [7] The characteristic radiological density of stone and an eccentric halo surrounding the area of low radiologic density as identified by

LubnaSamad et al and Garcia-Cuerpo et al is observed in32 (74.41%) cases in the present study. Garcia-Cuerpo et al. [4, 7]

The higher incidence of HBF has been thought to be due to the increasing usage of PCNL technique for stone disease. [4] This corroborates with our findings from the high endemic zone in India. [30] However, the peculiar findings of HBF in the present study need to be further evaluated for the hypothetical role played by the third type of renal stem cells. [17]

Conclusion

Although rarely reported in the literature, metaplastic bone formation has been found in relation to renal pelvis and proximal to PUJ. The frequency of heterometaplastic bone formation encompassing crystal deposits in our patients is unique and significantly higher. The pathogenetic mechanisms; calcium oxalate crystallization, Randall's plaques and stem cells of renal pelvis and papilla differentiating along bone lineage have been hypothesized. In depth analysis has opened up the unexplored avenue of epithelial-mesenchymal trans-differentiation of urothelial stem cells in HBF in renal stone disease. We corroborate these findings in our study.

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Financial or other Competing Interests: None.

Date of Submission : 11.04.2017
Date of Acceptance : 28.08.2017
Date of Publication : 18.12.2017