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SYMPOSIUM PAPER

Photmicrography of urinary deposits in stone clinic

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Abstract The importance of routine urine deposit study has not been projected satisfactorily in literature. This paper analyses the findings of urine microscopy of urinary stone patients who attended the stone clinic. A total number of 800 patients who attended the urinary stone clinic during the years 2005–2007 were selected for the study. Each patient had two samples of urine studied; early morning urine (EMU) and random. The patients were classified into different groups as proved stone patients (304), colic patients (289) and crystalluria patients (207). They were further classified as pre-treatment group and post-treatment group. The patients had chemotherapy depending on the biochemical abnormalities. The urine samples were centrifuged and the deposits examined under the low-power and high-power magnifications of the binocular microscope. The appropriate fields were photographed using a micro-photographic camera. 23% of the urinary samples studied contained deposits (36% of the EMU and 16% of the random samples). The most common deposits were red blood cells (RBC) (17%), pus cells (PC) (13%), calcium oxalate monohydrate (COM) crystals (7%), calcium oxalate dihydrate (COD) crystals (11%), uric acid crystals (2%), amorphous phosphates (1%), epithelial cells (13%) and sperms (7%). The unusual deposits included ammonium urate and cystine. Comparison of the results of deposits with those of 473 deposits from other laboratories showed that the present reports showed much more deposits than the outside ones. Deposits were more in the male patients (25%) compared to the females (19%). 83% of the patients with significant deposits had symptoms at the time of collection of sample, while 17% were not symptomatic. Among the patients with crystals, 53% had RBC associated and 49% had PC. RBCs were seen most in the COD crystal group. PC alone were seen in 2% and all were females. Percentage of urinary deposits was more in the pre-treatment group (32%) than in the post-treatment group (17%). Extent of crystalluria was more in the colic group (38%) compared to the crystalluria (22%) and stone (13%) groups. It is concluded from the study that accurate assessment of the urinary stone patient lies in a proper microscopic evaluation. It is mandatory that EMU should be examined as there is greater chance of identifying crystals and other deposits. Centrifuged deposits showed more deposits and these should be standards in urine examination. Regular urine deposit examination should be performed in all patients coming for follow-up.

Keywords Urinary deposit · Whewellite · Weddellite · Uric acid · Struvite · Brushite · Twinning · Aggregation · Clumping · Epithelial ghost · Crystal matrix interrelationship

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Introduction

Urinary stone disease is increasing in incidence during the last four decades. The problem may be stone, colic or crystalluria. Urinary deposits are closely related to the

formation of urinary stones. In present day clinical practice, the presence of crystalluria is hardly recognized. Crystalluria is a marker of urine super saturation and its examination is essential for detecting and following renal stone disease. A proper assessment of crystalluria pattern should recognize normal crystals (COD) in abnormal extent, abnormal crystals, large size, abundance, crystal aggregation and frequency of crystals present [1]. However, the study of these deposits is not routinely done in the stone clinics. Patients are unaware of the importance of this study periodically. This lacuna in the diagnostic set up of clinical system prompted the authors to make an in-depth study of urinary deposits of patients having suspected or proved urinary stone disease who attended the stone clinic.

Methods

A total number of 800 patients who attended the urinary stone clinic during the years 2005–2007 were selected for the study. The clinical and demographic data were recorded. 473 reports of deposits of patients who were studied elsewhere earlier were recorded and compared with the present results. Each patient had two samples of urine studied; early morning urine (EMU) and random samples. They were classified into different groups as proved stone patients (304), colic patients (289) and crystalluria patients (207), male and female, symptomatic and asymptomatic and pre-treatment group and post-treatment group. The patients had chemotherapy depending on the biochemical abnormalities. The urine samples were centrifuged and studied under light microscope at magnifications of 70 and 280 using Olympus binocular microscope. Significant deposits were photographed with Olympus 8.5 mega pixel camera and saved. The appropriate fields were photographed using a micro-photographic camera. Deposits studied included red blood cells (RBC), pus cells (PC), calcium oxalate monohydrate (COM), calcium oxalate dihydrate (COD), uric acid (UA), amorphous phosphate, triple phosphate, ammonium urate, epithelial cells and sperms. The number of crystals per high power field (HPF), their size, the presence of aggregation and clumping and possible crystal matrix interrelationships were recorded.

Results

23% of the urinary samples studied contained deposits. The most common deposits were RBC (17%), PC (13%), COM crystals (7%), COD crystals (11%), uric acid crystals (2%), amorphous phosphates (1%), epithelial cells (13%) and sperms (7%). The unusual deposits included ammonium urates and cystine. On performing a comparison of the results of urinary deposits of present study with those of 473 patients who had urine deposits studied from other laboratories prior to the attendance at the stone clinic, it was seen that the percentage of reported deposits was much lesser in the outside laboratories. None of the outside reports were recorded as EMU/random or centrifuged/uncentrifuged. 15.4% of the reports showed the presence of one or more deposits, namely RBC (20%), PC (23%), crystals (6.3%), which included COD (5%), UA (0.4%), amorphous phosphates (0.4%) and triple phosphate (0.4%). No report from outside labs mentioned about COM crystals (Table 1).

A more detailed study of the urinary deposits reported in the study revealed very interesting information. Deposits were more in the EMU (30%) compared to the 16% in the random samples (Table 2). Deposits were more in the male patients (24.9%) compared to the females (18.8%), as detailed in Table 3. 83% of the patients with significant deposits had symptoms at the time of collection of sample, while 17% were not symptomatic. Among 478 symptomatic patients, 48.3% had significant crystalluria as against 12.2% in 1,122 asymptomatic patients (Table 4). Among the patients with crystals, 53% had RBC associated and 49% had PC. RBC was seen most in the COD crystal group, compared to the COM, mixed crystals or uric acid alone group. PC alone group were 2% and all were females (Table 5). Percentage of urinary deposits was more in the pre-treatment group (32.2%) than in the post-treatment group (16.9%) as shown in Table 6. Extent of crystalluria

Table 1 Type of deposits in urine samples studied in stone clinic and elsewhere

S. no.	Item	Stone clinic (1,600)		Studied elsewhere (473)	
		Number	Percentage	Number	Percentage
1.	RBC	272	17.0	95	20.1
2.	PC	210	13.1	109	23.0
3.	COM	114	7.1	0	–
4.	COD	180	11.3	24	5.1
5.	UA	35	2.2	2	0.4
6.	Amorphous phosphates	14	0.9	2	0.4
7.	Triple phosphate	10	0.6	2	0.4
8.	Ammonium urate	15	0.9	0	–
9.	Cystine	3	0.2	0	–
10.	Epithelial cells	210	13.1	15	3.2
11.	Sperms	110	6.9	3	0.6
	Total	1173 (368)	23% of 1,600	252 (73)	15.4% of 473

Table 2 Type of deposits in early morning urine samples vs random samples

S. no.	Item	Early morning urine (800)		Random urine (800)	
		Number	Percentage	Number	Percentage
1.	RBC	210	26.3	62	7.8
2.	PC	154	19.3	56	7.0
3.	COM	71	8.9	43	5.4
4.	COD	128	16.0	52	6.5
5.	UA	26	3.2	9	1.1
6.	Amorphous phosphates	11	1.4	3	0.4
7.	Triple phosphate	7	0.9	3	0.4
8.	Ammonium urate	12	1.5	3	0.4
9.	Cystine	2	0.3	1	0.1
10.	Epithelial cells	137	17.1	73	9.1
11.	Sperms	94	11.8	16	2.0
	Total	852 (240)	30% of 800	321 (128)	16% of 800

Table 3 Type of deposits in male versus female urine samples

S. no.	Item	Male (1,406)		Female (186)	
		Number	Percentage	Number	Percentage
1.	RBC	246	17.5	26	7.8
2.	PC	182	12.9	28	7.0
3.	COM	96	6.8	18	5.4
4.	COD	160	11.4	20	6.5
5.	UA	29	2.1	6	1.1
6.	Amorphous phosphates	12	1.4	2	0.4
7.	Triple phosphate	9	0.9	1	0.4
8.	Ammonium urate	13	0.9	2	0.4
9.	Cystine	2	0.1	1	0.1
10.	Epithelial cells	180	12.8	30	9.1
11.	Sperms	110	7.8	0	0
	Total	1,039 (351)	24.9% of 1,406	134 (35)	18.8% of 186

Table 4 Type of deposits in symptomatic vs asymptomatic patients' urine samples

S. no.	Item	Symptomatic (478)		Asymptomatic (1,122)	
		Number	Percentage	Number	Percentage
1.	RBC	206	17.5	66	7.8
2.	PC	147	12.9	63	7.0
3.	COM	75	6.8	39	5.4
4.	COD	143	11.4	37	6.5
5.	UA	27	2.1	8	1.1
6.	Amorphous phosphates	3	1.4	11	0.4
7.	Triple phosphate	2	0.9	8	0.4
8.	Ammonium urate	3	0.9	12	0.4
9.	Cystine	2	0.1	1	0.1
10.	Epithelial cells	71	12.8	139	9.1
11.	Sperms	37	7.8	73	0
	Total	716 (231)	48.3% of 478	457 (137)	12.2% of 1122

Table 5 Relationship of RBC and pus cells to different crystals

S. no.	Item	RBC (272)		PC (210)	
		Number	Percentage	Number	Percentage
1.	COM male	51	18.8	43	20.5
2.	COM female	8	2.9	7	3.3
3.	COD male	91	33.5	66	31.4
4.	COD female	11	4.0	7	3.3
5.	UA male	26	9.6	19	9.1
6.	UA female	3	1.1	2	1.0
7.	Mixed, miscellaneous male	32	11.8	33	15.7
8.	Mixed, miscellaneous female	13	4.8	13	6.2
9.	No crystals males	28	10.3	17	8.1
10.	No crystals females	9	3.3	3	1.4
	Total	272		210	

was more in the colic group (38.1%) compared to the crystalluria (22.2%) and stone (13.2%) groups (Table 7). Table 8 details the special crystal characteristics namely number per high power field, size of crystals, crystal aggregation and crystal clumping. Largest crystals were uric acid followed by amorphous phosphates. Aggregation was most in uric acid crystals and clumping was most in COD.

Most commonly encountered urinary deposit was RBC, which was seen as round discrete appearances (Fig. 1). Figure 2 shows the larger WBCs interspersed between various crystals. Calcium oxalate monohydrate crystals (Fig. 3) appeared as oval crystals or as large typical dumbbell shaped (Fig. 4) ones. Sometimes the COM crystals were seen alone and at other times they were seen

Table 6 Type of deposits in pre treatment and post treatment groups

S. no.	Item	Pre treatment (466)		Post treatment (1,134)	
		Number	Percentage	Number	Percentage
1.	RBC	166	17.5	106	7.8
2.	PC	138	12.9	72	7.0
3.	COM	61	6.8	53	5.4
4.	COD	119	11.4	61	6.5
5.	UA	29	2.1	6	1.1
6.	Amorphous phosphates	7	1.4	7	0.4
7.	Triple phosphate	6	0.9	4	0.4
8.	Ammonium urate	12	0.9	3	0.4
9.	Cystine	2	0.1	1	0.1
10.	Epithelial cells	56	12.8	154	9.1
11.	Sperms	49	7.8	61	0
	Total	645 (150)	32.2% of 466	528 (192)	16.9% of 1134

along with COD crystals. The size of the COM crystals was variable and in some cases, the crystal size was very large (Fig. 4). Aggregation of COM crystals (Fig. 5) was seen in some deposits and in certain other samples clumping of crystal (Fig. 6) along with organic materials was noted. COD crystals were seen as typical envelope shaped ones (Fig. 7). Many patients had associated RBCs along with COD crystals (Fig. 8). The size was varying in the same field. Some of the COD crystals were very large (Fig. 9) indicating severe crystal pathology. Crystal twinning (Fig. 10) was seen along with larger-sized COD crystals.

Table 8 Special crystal characteristics in deposits studied

S. no.	Item	Number/ HPF	Size (μm)	Crystal aggregation (%)	Crystal clumping (%)
1.	COD	8	18	5	17
2.	COM	5	12	3	8
3.	UA	3	59	13	12
4.	Amorphous phosphate	1.5	38	5	3
5.	Triple phosphate	1.2	42	3	2
6.	Ammonium urate	12	16	0.5	–
7.	Cystine	1	27	–	–

Aggregation of COD crystals (Fig. 11) and crystal clumping in association with organic matrix (Fig. 12) were also seen. Uric acid crystals showed very interesting findings. Many patients had large rhomboidal crystals (Fig. 13) and in some cases, barrel-shaped crystals were seen in large numbers (Fig. 14). Sometimes very large crystals were seen in urine (Fig. 15). Uric acid crystals were seen either alone or along with COM crystals (Fig. 16). Twinning was seen as incorporation of small crystals getting attached to larger rhomboidal crystals (Fig. 17). Aggregation was seen more in rhomboidal crystals (Fig. 18). Clumping was seen in some patients with large masses of matrix engulfing the uric acid crystals (Fig. 19). Ammonium urate crystals were slightly larger than the WBCs and had a dense border (Fig. 20). Crenations were seen on close observation. Ammonium urate also showed aggregation in some patients (Fig. 21). Struvite crystals were not very common and were occasionally seen as coffin lid shaped (Fig. 22). Brushite crystals were seen mostly in columns (Fig. 23).

Table 7 Type of deposits in stone, colic and crystalluria patients

S. no.	Item	Stone (304)		Colic (289)		Crystalluria (207)	
		Number	Percentage	Number	Percentage	Number	Percentage
1.	RBC	22	7.2	126	43.6	124	59.9
2.	PC	17	5.6	112	38.8	81	39.1
3.	COM	18	5.9	51	17.7	45	21.7
4.	COD	33	10.9	89	30.8	58	28.0
5.	UA	7	2.3	19	6.6	9	4.4
6.	Amorphous phosphates	2	0.7	3	1.0	9	4.4
7.	Triple phosphate	2	0.7	3	1.0	5	2.4
8.	Amorphous urate	4	1.3	3	1.0	8	3.9
9.	Cystine	0	0.0	2	0.7	1	0.5
10.	Epithelial cells	31	10.2	76	26.3	83	40.1
11.	Sperms	32	10.5	35	12.1	43	20.1
	Total	278 (40)	13.2% of 304	519 (110)	38.1% of 289	134 (46)	22.2% of 207

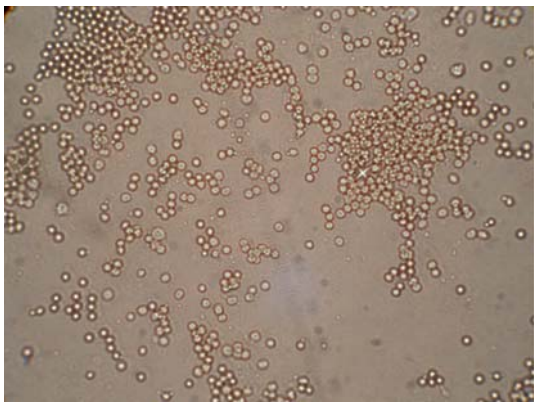

Fig. 1 RBC in urinary deposit

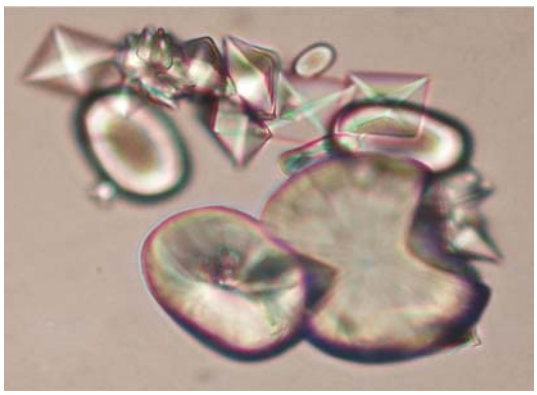

Fig. 4 Large dumbbell shaped COM crystals

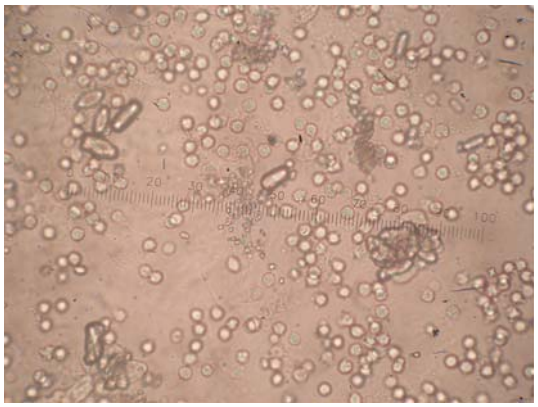

Fig. 2 Pus cells (WBC) in deposit

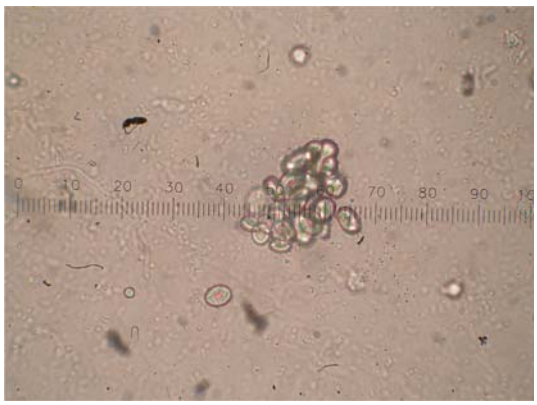

Fig. 5 COM crystal aggregation

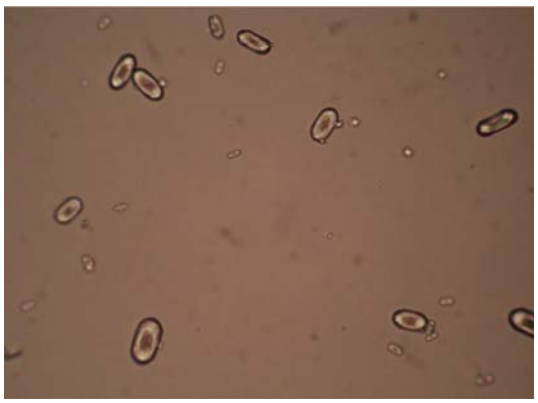

Fig. 3 Oval COM crystals

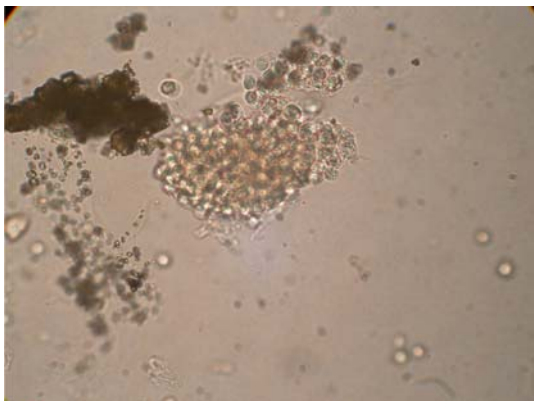

Fig. 6 COM crystal clumping

Amorphous phosphates were irregular and large (Fig. 24). Carbonate apatite was fairly uncommon but occasionally seen along with struvite crystals (Fig. 25). Cystine crystals were rarely encountered (Fig. 26) in hexagonal shape. Epithelial cells were in abundance in many patients, but not associated with significant crystalluria (Fig. 27). However, dismembering epithelial cells appeared to attract crystals and presenting as epithelial ghosts (Fig. 28). Organic matrix as such was seen in a fair number of deposits (Fig. 29) and in certain areas, crystals got embedded within them (Fig. 30) to represent crystal matrix interrelationships.

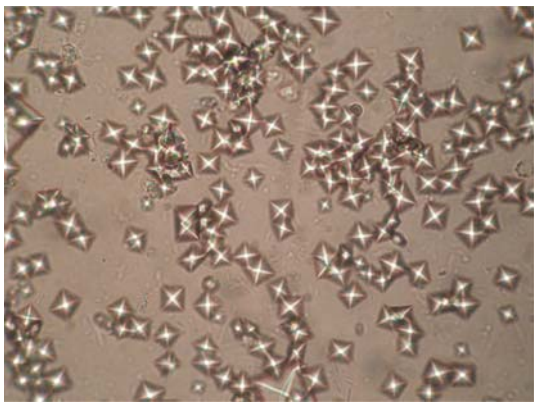

Fig. 7 COD crystals

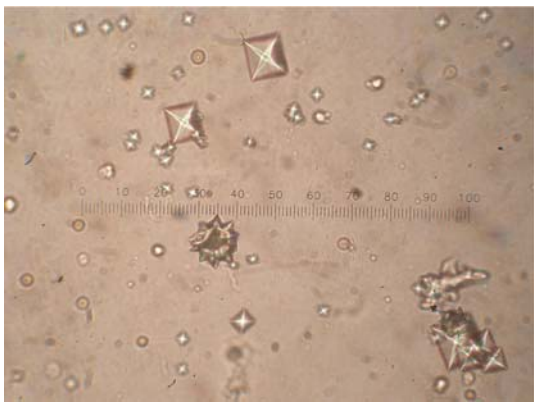

Fig. 10 COD crystal twinning

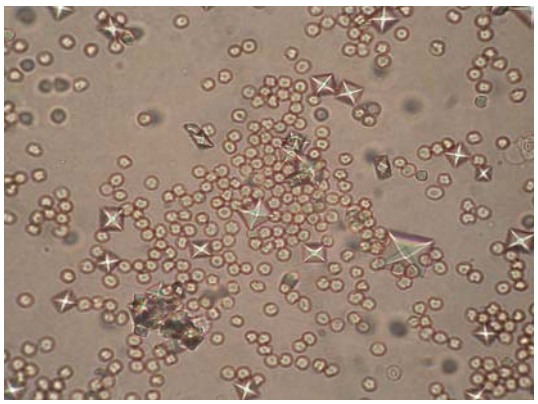

Fig. 8 COD large crystals + RBC

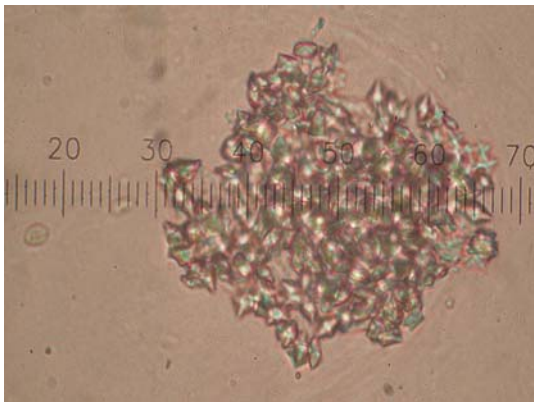

Fig. 11 COD crystal aggregation

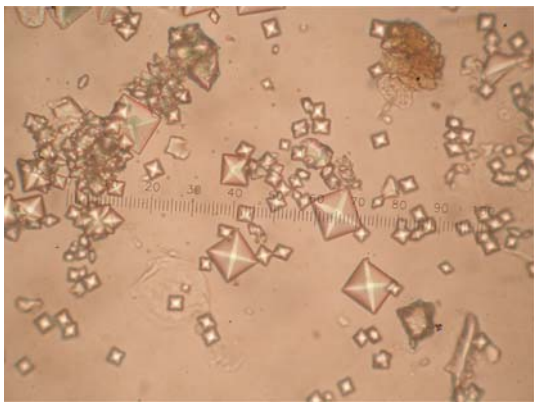

Fig. 9 COD large crystals

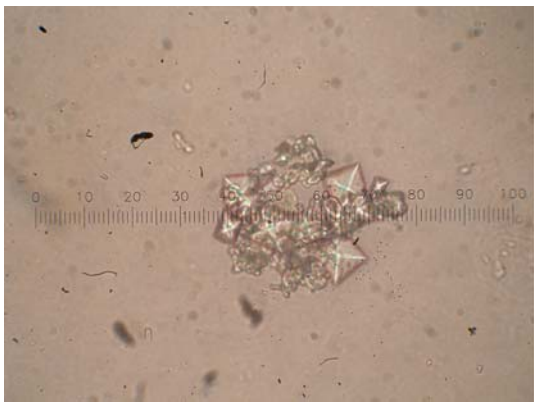

Fig. 12 COD crystal clumping

Discussion

Significant crystalluria is a pathology in many patients suffering from urinary stone disease. Many patients visit the orthopedic surgeon for low back ache. This paper highlights the pitfalls in recognition of important crystals in clinical laboratories. The high percentage of crystalluria stresses upon the need for recognizing crystals such as COM, which are rarely reported by clinical laboratories. In this paper, the different samples, namely EMU and random were studied as separate entities and so the percentage of samples with crystalluria appears lower. A patient's urinary deposit will be considered pathological when significant

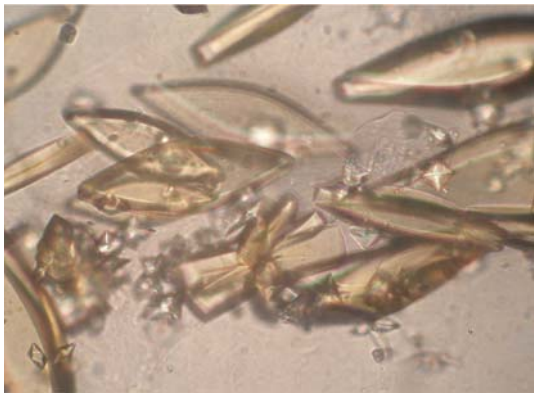

Fig. 13 Rhomboidal uric acid crystals

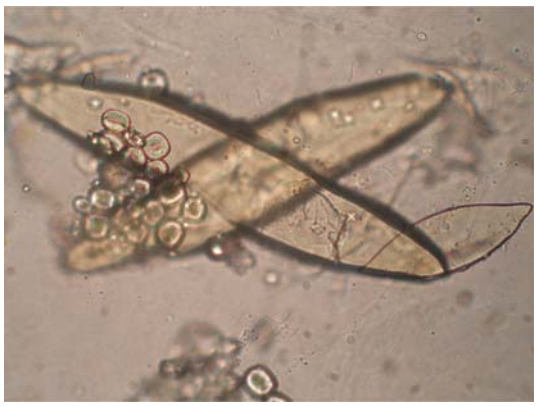

Fig. 16 Uric acid + COM crystals

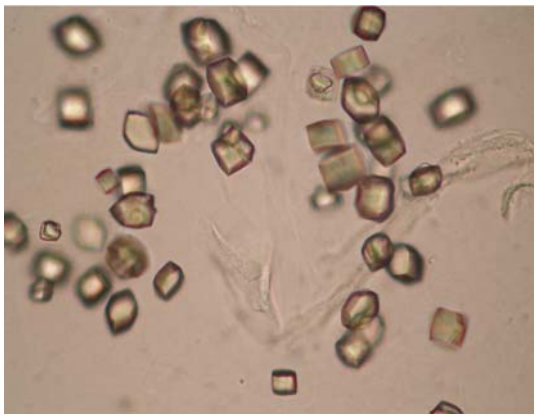

Fig. 14 Barrel shaped uric acid crystals

Fig. 17 Uric acid twinning

Fig. 15 Large uric acid crystal

Fig. 18 Uric acid aggregation

crystalluria is recognized in any of the samples passed by the patient during any time of the day. On combining the findings of the EMU and random samples, the percentage of urinary deposits will become much higher. Significant deposit in this paper was considered only when the number per high power field was more than 3. This decision might have excluded some reports of deposits, which might have been clinically relevant.

Calcium oxalate is formed in the kidney as calcium oxalate trihydrate. When the crystals pass through the collecting tubules, they are dehydrated to form calcium oxalate dihydrate crystals. We have recognized that 12% of

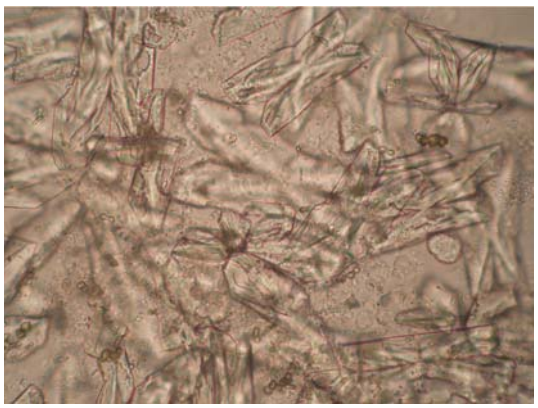

Fig. 19 Uric acid clumping

Fig. 22 Struvite crystals

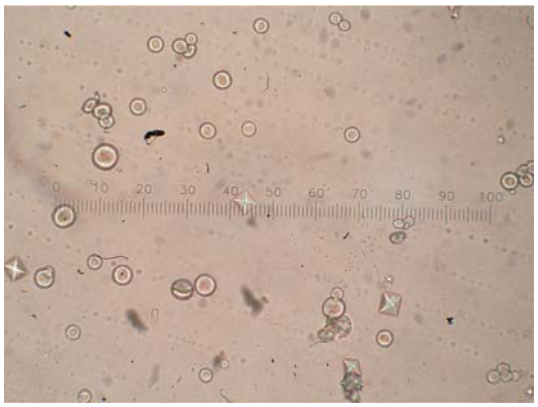

Fig. 20 Ammonium urate crystals

Fig. 23 Brushite crystals

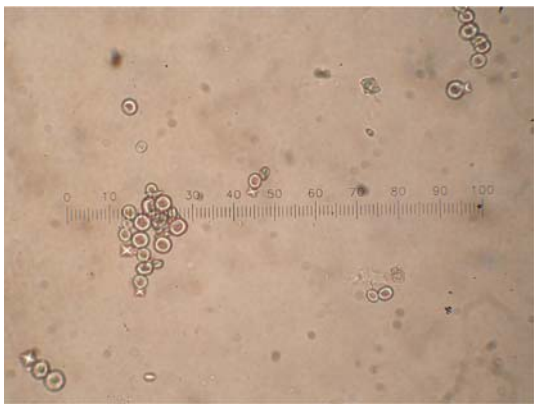

Fig. 21 Ammonium urate aggregation

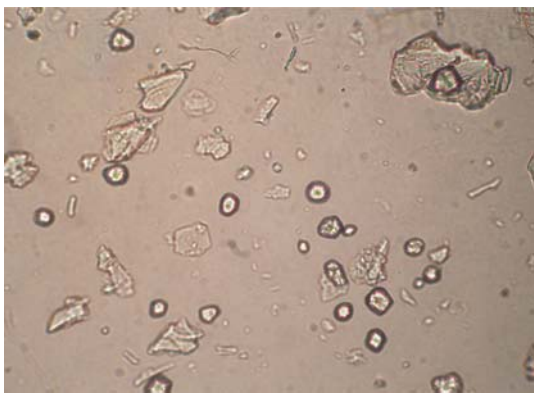

Fig. 24 Amorphous phosphate crystals

the normal population may have COD crystals in urine at some time or the other. In stone patients, however, the COD crystals get dehydrated to form COM crystals. COM crystals are more stable and are considered pathological indicating active stone formation. Review of literature shows that various authors have done extensive work on different types of stone disease and stressed on the clinical relevance of deposit findings in disease formation. The presence of cystinuria has been reported to be important in formation of cystine calculi [2]. The crystal pattern obtained from the bladder urine and renal urine has been reported to be different [3]. Some believe that the urine samples maintained at 37°C after collection will provide a clear picture regarding the extent of crystalluria [4, 5]. The

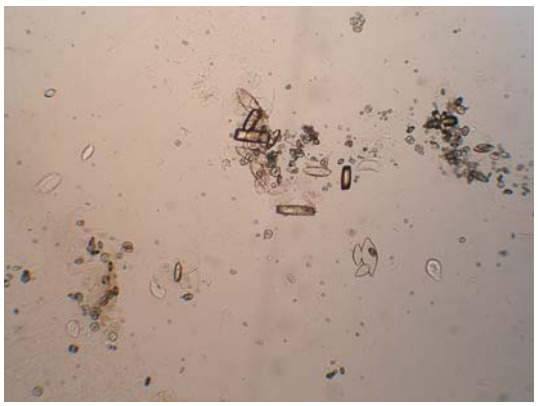

Fig. 25 Carbonate apatite crystals

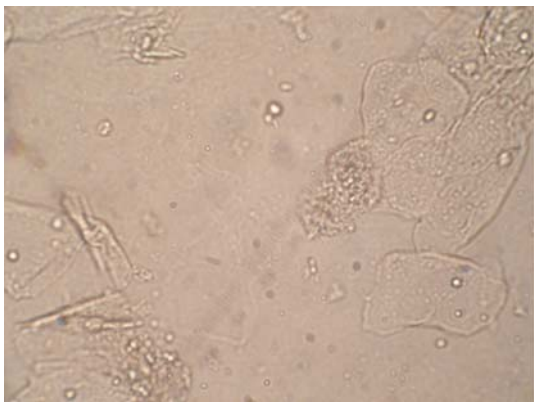

Fig. 28 Epithelial ghosts

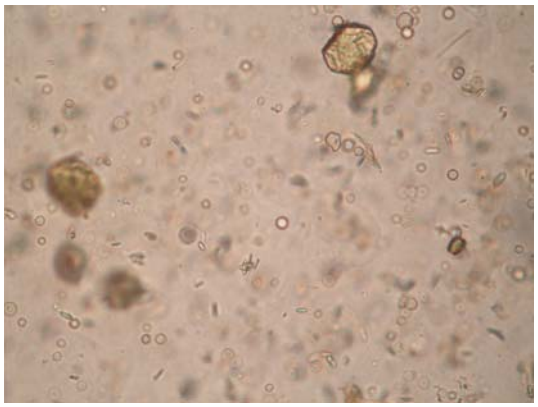

Fig. 26 Cystine crystals

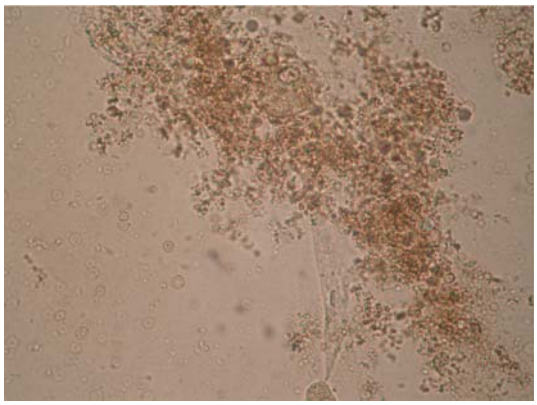

Fig. 29 Organic matrix

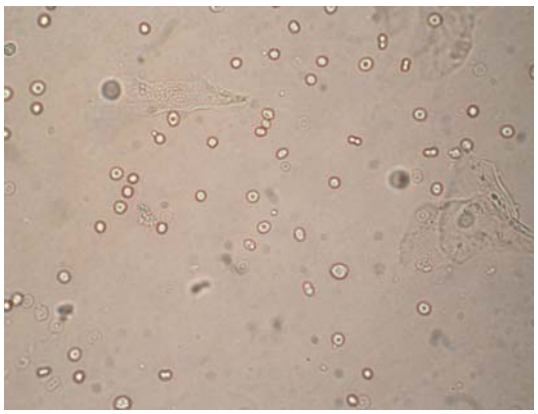

Fig. 27 Epithelial cells

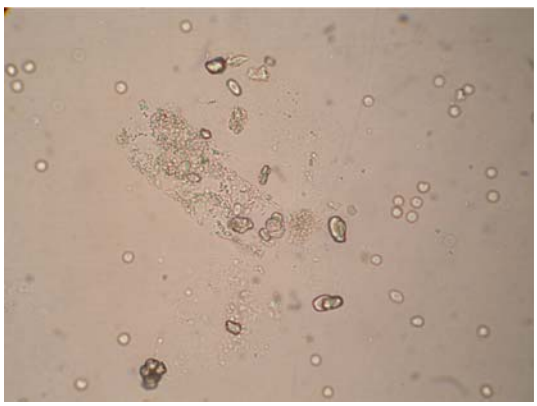

Fig. 30 Crystal matrix interrelationship

extent of crystalluria has been compared with urine and blood metabolic studies. Correction of metabolic abnormalities will be corresponding to the inhibition of crystal deposition [6]. The presence of pus cells in the urine is highlighted. Only 3% of urine samples with pus cells in urine was positive in culture. Pus cells reported in the urine deposit may be due to infection or may be WBC as a part of the blood in the urine produced by injury to the urothelium by a moving stone or crystal. This aspect is not considered by the clinicians before administering antibiotics to such patients. We have done a histochemical study of urine deposits, which showed that WBC of trauma had regular cell outline whereas pus cells produced by infection

had crenated appearance. Recognition of this fact can avoid injudicious administration of antibiotics. Uric acid crystals are not commonly reported in usual laboratory tests. Sodium urate and ammonium urate crystals are still rarely reported.

Crystal clumping and aggregation are other features not usually reported in urinary deposits. Agglomeration of calcium oxalate crystals is an indicator of stone formation and this should be studied to identify the effect of treatment [7]. Aggregation of crystals means the crystals joining together to form larger crystals. Aggregation has been reported only in calcium oxalate stone formers and not in normals [5, 8]. Clumping of crystals means the entanglement of crystal in protein matrix. Some authors believe that the first step in stone formation is a nucleus of organic colloid materials in the tubules or calyx [9, 10]. Modulators of urinary stone formation influence the crystallization in tubular urine, crystal retention in tubules and plaque formation in the nephron. It is believed that inhibitory activity of crystal growth is produced by glycoproteins and glycosaminoglycans [11] and other macromolecules like nephorocalcin, osteopontin (uropontin), uronic acid rich protein and others [12]. This indicates that patients with aggregation and clumping should be followed up to look for the reduction in extent of these during follow-up. The size of the crystals matters significantly in the development of stone disease. Unfortunately the size is never reported in usual reports. Stone formation is higher in patients with larger size of crystals. This is particularly relevant for COD crystals.

It is obvious from the observations of this study that proper recording, analysis and interpretation of the urinary deposits of stone patients are essential for proper understanding of possible initiation of stone disease and monitoring the effect of therapy by scientific assessment of the crystal pattern of the urine of stone formers [8, 13] during follow-up and predict the risk of future stone formation, so that appropriate chemotherapy and chemoprophylaxis can be administered to the patients scientifically.

Conclusion

It is concluded from the study that study of urine deposit of stone patients is of paramount importance in establishing the pathology of stone disease. An accurate assessment of the urinary stone patient lies in a proper microscopic evaluation. It is mandatory that EMU should be examined as there is greater chance of identifying crystals and other deposits. Centrifuged deposits showed more extent of deposits and these should be standards in urine examination. Regular urine deposit examination should be performed in all patients coming for regular follow-up to study the size of the crystals, the presence of COM and unusual crystals like sodium urate, ammonium urate, cystine and the presence of crystal aggregation and clumping, epithelial ghosts and possible crystal matrix interrelationships.

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