PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE 168, 254–258 (1981)

Serum Phosphate Abnormalities in Sickle Cell Anemia (41269)

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Abstract. Persistent or intermittent elevation of serum phosphate levels was observed in five adults with sickle cell anemia. Since this may be clinically relevant, possible causes for the high phosphate levels were examined and the association with painful crises was evaluated. Increased serum phosphate levels were regularly associated with increased renal tubular reabsorption of phosphate. Normal parathormone levels and normal renal responses to parathormone were observed in this group and no change in the serum phosphate level or in the renal tubular defect was noted after transfusion to a normal hemoglobin level. Increased renal clearance of sodium was also observed in this group. The findings show that increased levels of serum phosphate in patients with sickle cell anemia are related to altered renal handling of phosphate which is associated with increased clearance of sodium. The frequency of crises was markedly increased in the patients with high levels of serum phosphate.

Increased levels of serum phosphate may occur for several reasons. These include abnormalities in the renal handling of phosphate, disorders associated with excessive catabolism, and alterations in endocrine function as observed in hypoparathyroidism. Modest increases in serum phosphate levels significantly affect red cell metabolism, increase 2,3-diphosphoglycerate (DPG) levels, and cause decreased affinity of oxygen for hemoglobin, i.e., a rightward shift of the hemoglobin–oxygen dissociation curve (1). We have confirmed the finding by De Jong and co-workers (2) that some adults with sickle cell anemia have persistent or intermittent hyperphosphatemia. Such changes could have significant effects on these patients since a rightward shift in the hemoglobin–oxygen dissociation curve would be associated with decreased oxygen saturation and an increased sickling of red cells (3) as well as with shortening of the delay time for hemoglobin polymerization (4). These changes could be related to more frequent vasocclusive events. Because of the potential importance of this finding we have examined possible causes for it. We have also determined the frequency of vasocclusive crises in these patients as compared to normophosphatemic adults with sickle cell anemia.

Methods. Patients between the ages of 18 and 42 years with sickle cell anemia confirmed by clinical findings, hemoglobin electrophoresis, and family studies when indicated were evaluated. The patients were long-term patients of the Hematology Unit (24–206 months, mean 100 months) and were regularly cared for on the Unit. Blood counts and hemoglobin electrophoresis were done by standard methods. None of the patients showed significant or active bone disease. Transfusion was done with fresh compatible packed red cells (Hb AA). Seven healthy black hospital employees without sickle cell disease were control subjects.

Renal studies were done while the patients were clinically stable and had unchanging blood counts, electrolytes, and glomerular filtration rates (GFR). The patients were given nothing by mouth after midnight and urine was collected from 8 AM to noon, for determination of calcium, phosphorous, creatinine, and sodium. This was preceded by 3 days of a general hospital diet supplemented with three to four glasses of milk daily. Similar tests were performed upon blood drawn at 10 AM. Measurements in both serum and urine included calcium determined by atomic absorption spectrophotometry, phosphorous

0037-9727/81/100254-05$01.00/0

and creatinine by autoanalyzer, and sodium by flame photometry (5). Serum phosphate levels were generally the average of two determinations done at a 1-hr interval. Urine volumes per minute were corrected to 100 ml/min GFR, using the creatinine clearances as the measurement of GFR. Tubular reabsorptive capacity for phosphate ($T_{m,p}$/GFR) was calculated by the formula $T_{m,p}/GFR = [(S_p \times Cl_{cr}) - (U_p \times V)] \div Cl_{cr}$, S_p = serum phosphate (mg/dl), Cl_{cr} = creatinine clearance (ml/min), U_p = urine phosphate (mg/dl), and V = urine flow (ml/min, uncorrected). Sodium clearances were determined by multiplying the urine concentration by the corrected urine volume and dividing by the respective serum concentration. PTH levels were determined by radioimmunoassay (Upjohn Clinical Laboratory, Kalamazoo, Mich.) during the preinfusion period in the three patients with increased phosphate levels and sickle cell anemia. Urinary cyclic AMP was determined on 2 consecutive days, before and following PTH infusion in two patients with sickle cell anemia and high phosphate levels. On the first day of testing 10 ml of sterile water was infused by Harvard pump over a 45-min period. On the second day the procedure was repeated using 300 units of PTH (3 ml) mixed with 7 ml of sterile water in place of the 10 ml of sterile water used on the previous day. The PTH used on all patients was from the same lot. Urinary cAMP was determined by radioimmunoassay (Bio-Science Laboratory, Van Nuys, Calif.).

The criteria used to determine which crises should be included to evaluate crisis frequency were described earlier (6). We used Grade 2 level painful crises, i.e., those crises requiring hospitalization, in this study. The frequency of crises was determined by dividing the total number of crises by the number of months of observation.

Results. Renal handling of phosphate was studied in nine patients with sickle cell anemia and in seven normal subjects (Table I). Five patients had elevated serum phosphate levels and four had serum phosphate levels within the normal range (3.0–4.5 mg/dl). Increased serum phosphate levels were associated with significantly higher tubular reabsorptive capacities for phosphate as compared to results in patients with normal serum levels and to normal controls. The statistical differences were shown by one-way analysis of variance and by Duncan's multiple comparison test at the $\alpha = 0.05$ level. In patient 4, transfusion with packed cells to a normal hemoglobin level was not associated with a decrease of renal phosphate reabsorption or a change in serum phosphate level. Maximal tubular reabsorptive capacity for phosphate in the patients with normal serum phosphate levels and in the control subjects did not differ.

The increase in tubular reabsorptive capacity of phosphate observed in the high phosphate group of patients was accompanied by an increase in renal sodium clearance when compared to sodium clearance in patients with normal phosphate levels. This was statistically significant as shown by analyses similar to those described above. The mean sodium clearance in the patient controls ($M = 0.18$ ml/min/100 ml GFR) with normal phosphate levels was lower than that of normal controls ($M = 0.27$ ml/min/100 ml GFR), however, the difference was not statistically significant. The relationship of serum phosphate to age in these patients was not significant ($r = 0.13$, $P > 0.05$).

PTH infusion studies were performed in three patients with sickle cell anemia and an increased serum phosphate. During the infusion in the third patient, pain occurred simulating possible crisis pain and the study in this patient was terminated. In view of the known effect of PTH on acid–base balance, it was decided not to use this hormone on any other patient with sickle cell anemia. The results of PTH infusion upon the first two patients with sickle cell anemia and elevated phosphate levels appear in Table II. PTH evoked a marked rise in the urinary excretion of cAMP in both patients.

PTH levels in three patients with elevated serum phosphate levels and sickle cell anemia who were studied were 467, 295, and 208 pg/ml with serum calcium levels of 9.8, 8.6, and 9.4 mg/dl, respec-

TABLE I. STUDIES ON PATIENTS WITH SICKLE CELL ANEMIA AND VARYING SERUM PHOSPHATE LEVELS

Patient (sex)	Age (years)	Hemoglobin (g/dl)	Serum phosphate[a] (mg/dl)	Urine volume (ml/min)	Creatinine clearance (ml/min)	Urine phosphate (mg/dl)	Urine sodium (meq/l)	$T_{m,p}$/GFR[b]	Sodium clearance (ml/min/100 ml GFR)
				Patients—High serum phosphate					
1 (F)	27	6.4	5.6	0.85	97	27.94	80	5.36	0.51
2 (M)	26	9.2	4.7	1.69	201	17.12	110	4.56	0.62
3 (F)	42	8.8	4.7	1.46	140	4.34	60	4.66	0.45
4 (F)[c]	22	8.9	4.7	1.46	91	11.57	46	4.51	0.55
4 (F)[d]	22	15.5	4.7	2.46	149	28.00	61	4.24	0.73
5 (F)	24	8.4	4.6	2.58	173	31.89	21	4.12	0.23
				Patients—Normal serum phosphate					
1 (F)	26	8.2	4.2	0.88	144	51.16	45	3.89	0.20
2 (F)	20	7.8	4.0	1.04	148	44.00	48	3.69	0.24
3 (M)	37	9.6	4.0	4.58	154	27.74	10.2	3.18	0.22
4 (F)	21	6.0	3.7	1.75	221	20.88	12	3.54	0.07
				Normal controls					
1 (F)	30	14.5	4.1	0.33	91	85.42	20	3.79	0.05
2 (M)	22	13.8	3.8	1.14	93	46.00	87	3.24	0.75
3 (M)	34	14.0	4.5	1.66	74	18.60	13	4.08	0.21
4 (M)	27	13.7	3.1	1.39	118	40.19		2.63	
5 (M)	30	15.4	3.8	0.83	135	61.00	8	3.43	0.35
6 (M)	26	16.9	2.9	0.52	134	78.70	7	2.60	0.02
7 (M)	26	14.7	3.8	5.40	146	11.30	8	3.38	0.21

[a] Normal serum phosphate (3.0–4.5 mg/dl).
[b] Tubular reabsorptive capacity for phosphate (calculated as described under Methods).
[c] Studies done before transfusion.
[d] Studies done after transfusion.

TABLE II. URINARY EXCRETION OF CYCLIC AMP BEFORE AND AFTER PARATHORMONE INFUSION

	Patient 1		Patient 2	
	Day 1	Day 2[a]	Day 1	Day 2[a]
Serum calcium (mg/dl)	9.6	10.0	9.9	10.5
Serum phosphate (mg/dl)	5.6	5.1	4.7	4.0
Serum creatinine (mg/dl)	0.5	0.5	0.4	0.6
Urine cAMP (μmole/l)	4.0	23.0	2.3	10.0
Urine volume (ml/240 min)	203	340	406	507

[a] With PTH.

tively. Although the PTH level of one patient was elevated (467 pg/ml), the other two PTH levels were within normal limits for the level of serum calcium.

Crisis frequency was examined in 24 patients who were followed from 24 to 206 months (mean 100 months). Ten of these patients had elevated serum phosphate levels more often than normal levels during the period of observation and were designated as a high phosphate group. The 14 remaining patients had persistent normal phosphate levels and were designated as a normophosphate group (Table III). Using a two-sample t-test, the high phosphate group showed 0.22 ± 0.03 (SE) crisis frequency and the normal phosphate group and 0.06 ± 0.01 (SE) crisis frequency ($P < 0.001$).

Discussion. Our study shows that increased serum phosphate levels occur in some adults with sickle cell anemia. There appears to be a relationship between increased levels of serum phosphate and increased tubular reabsorption of phosphate. Tubular reabsorption of phosphate was determined by measuring $T_{m,p}$/GFR. This corrects for variations in phosphate reabsorption related to changes in filtered load of phosphate (7). The alteration in the tubular reabsorption of phosphate does not appear to be related to parathormone effects since neither hypocalcemia nor decreased levels of parathormone were present. The increase in urinary cyclic AMP excretion following parathormone infusion suggests that the tubular defect is probably not due to end organ insensitivity (8). Anemia, per se, was not a likely cause for the increased level of serum phosphate or the renal tubular abnormality since neither the serum phosphate level nor the renal defect were altered by transfusion to a normal hemoglobin level in the one patient so studied (No. 4). The defect in the tubular reabsorption of phosphate which we have observed is similar to observations made by De Jong and co-workers (2) We have extended the study to include normophosphatemic controls, parathormone studies, the response of the tubular defect to trans-

TABLE III. FREQUENCY OF PAINFUL CRISES IN PATIENTS WITH SICKLE CELL ANEMIA AND VARYING PHOSPHATE LEVELS

Normal phosphate		High phosphate	
Patient	Crisis frequency[a]	Patient	Crisis frequency[a]
1	0.18	1	0.22
2	0.06	2	0.19
3	0.10	3	0.29
4	0.04	4	0.09
5	0.07	5	0.44
6	0.07	6	0.21
7	0.06	7	0.15
8	0.02	8	0.17
9	0.03	9	0.16
10	0.06	10	0.26
11	0.02		
12	0.09		
13	0.04		
14	0.01		

[a] Crisis frequency was obtained by dividing the total number of crises by the number of months of observation.

fusion, and the relationship of the tubular abnormality to sodium clearance.

Patients with higher serum phosphate levels had an increase in the renal clearance of sodium. The change in sodium clearance may explain the increase in renal tubular phosphate reabsorption since increased sodium clearance may lead to total body sodium depletion and subsequent stimulation of proximal tubular reabsorption of phosphate. A relationship between phosphate and sodium clearances had been previously described although the findings are conflicting. Phosphaturia as well as naturiuresis was shown to occur acutely after saline infusion (9); parathormone has been demonstrated to reduce proximal tubular reabsorption of both ions (10). In contrast, a decreased clearance of phosphate and an increased clearance of sodium after relief of ureteral obstruction was demonstrated by Better *et al.*, (11). The latter association is similar to that seen in our patients. This similarity could be an expression of interstitial changes seen in the kidneys of patients with sickle cell anemia and obstructive nephropathy.

Of interest was the increased frequency of painful crises in the patients with elevated serum phosphate levels as compared to those with normal phosphate levels. The reason for this is not clear. It may be related to the effects of serum phosphate on red cell DPG metabolism, the associated rightward shift of the hemoglobin–oxygen dissociation curve, the decrease in oxygen saturation, and the subsequent enhancement of sickling of red cells (1, 2). The increased sensitivity of hemoglobin gelation to increased deoxyhemoglobin concentrations may also be a significant factor (3). The lower serum phosphate which occurs in normal adults as compared to children with its subsequent effect on hemoglobin–oxygen dissociation (1) may explain the decrease in crisis frequency in adults with sickle cell anemia as compared to children (12). Although the vasocclusive episodes in the present study only included painful crises and did not include other vasocclusive events, such as the acute chest syndrome, stroke or aseptic necrosis, none of these latter types of abnormalities were present in this group of patients. The differences in frequency of the painful crises between the group with increased phosphate levels and the group with normal levels are so marked that evaluation of serum phosphate level requires consideration as a method for predicting crisis frequency in patients with sickle cell anemia.

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Received May 26, 1981. P.S.E.B.M. 1981, Vol. 168.