

INCIDENCE OF RENAL ACIDIFICATION DEFECT IN UROLITHIASIS AS MEASURED BY AMMONIUM CHLORIDE LOADING TEST

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ABSTRACT

BACKGROUND

Urinary calculus is well known from ancient times and prevalent in several pockets of India and in Rajasthan also. The involvement of parathyroid hormone and renal tubular acidification defect in calculogenesis have not yet investigated properly in these particular region.

MATERIALS AND METHODS

The present study was planned with the following two objectives in view (a)–To study the incidence of Renal Tubular Acidification (RTA) defects and Hyperparathyroidism (HPT) in the local stone formers by using the short ammonium chloride loading test; and (b) To establish the adequate dose of ammonium chloride for the test. For the present study, patient of urinary calculous radio-opaque shadow were taken from AIIMS surgical ward and result compared with normal subject.

RESULTS

It was evident that 75 mg per kg body weight NH₄CL is inadequate dose, as only 50% of normal subject achieve pH up to 4.5 as compared of 100% on 150 mg per kg body weight of NH₄CL. After adequate challenge (150 mg ammonium chloride per kg body weight), all normal subject achieve pH 4.5, whereas in stone former only 46.2% could lower pH between 4.6 to 5 and 53.8% could not lower it below 5. This observation suggested the presence of incomplete RTA defect in 53.8% of stone formers. None of the subject excreted alkaline urine indicating absence of complete RTA defect. Ammonium chloride loading causes increased urinary calcium excretion in stone former as well as in normal subject no significant difference was observed and did not show any evidence of hyperparathyroidism in the subjects investigated.

CONCLUSION

Incomplete RTA defect present in 53.8% of stone formers investigated. Complete RTA defect not present in any stone formers. Hyperparathyroidism was not present in any of the subject. Short ammonium chloride loading test is a reliable tool to evaluate the presence of hyperparathyroid's and Renal Tubular Defect (RTA defect). For proper result, 150 mg/kg body weight ammonium chloride is adequate dose.

KEYWORDS

Renal Tubular Acidification [RTA] Defect [Complete and Incomplete], Hyperparathyroidism, Urinary Calculus Disease

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INTRODUCTION

Urinary calculus is well known from ancient times and prevalent in several pockets of India including Rajasthan and recurrence is also another disquieting feature. The involvement of parathyroid hormone and renal tubular acidification defect in calculogenesis has not yet investigated properly. As it has been stressed that hyperthyroidism must be considered in every patient presenting with renal tract stone or nephrocalcinosis and even in the cases of renal colic. Hyperparathyroidism is directly proportional to nephrocalcinosis, but measurement of H. P. T. is costly. So short ammonium chloride loading test is to ascertain parathyroid status is relatively less cumbersome and requires a very simple technique. Incidentally, this test can also be used as a diagnostic aid in renal tubular acidification

acidification defect resulting in the failure of renal tubules to secrete H⁺, which is often associated with failure to produce NH₄⁺ in the kidney resultantly urine become alkaline. It is associated with increased calcium and decreased potassium excretion, the higher excretion of calcium in alkaline urine tends to form calciferous stones with phosphates.

Some patient with nephrocalcinosis could not secrete highly acidic urine when challenged with an oral load dose 100 mg/kg body weight is given. Short NH₄CL loading test did not induce hypercalcemia in normal subject, but produce profound hypercalcemia in stone former patient suffering from HPT. RTA defect in stone formation produce chronic metabolic acidosis is associated with reduced renal tubular reabsorption of calcium result in hypercalciuria (Parfitt et al 1974¹).

The Present Project was Undertaken

- To examine the usefulness of different doses of NH₄CL [75 and 150 mg/kg body weight] for the assessment of H.P.T. and renal tubular acidification status.
- To investigate the involvement of hyperparathyroidism in stone formers of this region.
- To investigate the involvement of renal tubular acidification defect in stone formers of this region.

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MATERIAL AND METHODS

The present study was planned with the following two objectives in view (a) To study the incidence of Renal Tubular Acidification (RTA) defects and Hyperparathyroidism (HPT) in the local stone formers by using the short ammonium chloride loading test; and (b) To establish the adequate dose of ammonium chloride for the test.

40 radiologically proved cases of upper urinary tract stone disease (SF) and 20 healthy and Normal Subjects (NS) formed the case material for the study. Including male and female of 13 to 60 years and all kept on normal diet.

Sl. No.	Nature of Subjects	Range	Mean	SD+-	SE
1	Normal Subjects No. 20	16-15 Years	36.75	13.43	3.89
2	Stone Formers No. 40 Male 32 Female 8	12-60 Years 14-30 Years	34.76	14.11	2.82

Table 1: Mean Age of Selected Subjects

2 gm and 1 gm of elemental calcium (Ostocalcium) orally given on 2nd and 3rd days, the 24 hours urine sample collected on all three days and labelled as I, II and III. The three twenty four hours samples were analysed for calcium, inorganic phosphorus and creatinine and the values were calculated in the form of mg%, mg/24 hours, mg/gm of creatinine and mg/m² of BSA.

OBSERVATIONS

Sample No.	24 Hours	mg%	mg/24 Hours	mg/gm of Creatinine	mg/sq. m. BSA
1 First Day Normal Diet	Range	2.85-19.0	31.3-323.0	77.3-353.4	21.18-219.1
	Mean	10.5	155.2	195.0	103.0
	SD+ -	4.33	87.8	91.2	54.55
2 Second Day Normal Diet + 2 gm Calcium	Range	2.50-30.9	43.5-361.8	90.4-1208	32.2-247.0
	Mean	13.28	176.0	353.2	115.81
	SD	7.96	101.75	299.4	66.57
3 Third Day Normal Diet + 1 gm Calcium	Range	2.7-31.4	51.7-318.4	110.3-416.6	46.1-218.0
	Mean	12.67	164.35	255.2	106.46
	SD	8.51	90.3	104.0	54.33

Table 2: 24-Hour Urinary Excretion of Calcium in Normal Subjects on Normal Diet and with Calcium Supplementation

Sample No.	24 Hours	mg%	mg/24 Hours	mg/gm of Creatinine	mg/sq. m. BSA
1 First Day Normal Diet	Range	3.3-18.1	29.9-231.2	26.6-385.3	17.2-127.8
	Mean	8.73	103.8	167.33	69.96
	SD	4.19	57.9	88.94	37.08
2 Second Day Normal Diet + 2 gm Calcium	Range	2.5-22.5	33.75-302.5	57.7-503	22.5-213.4
	Mean	9.36	148.2	240.1	97.8
	SD	4.8	85.2	118.5	56.6
3 Third Day N.D. + 1 gm Calcium	Range	2.5-19.0	30.8-337.0	56.4-528.0	22.3-189.3
	Mean	9.8	138.3	221.55	92.0
	SD	5.25	57.1	102.77	47.14

Table 3: 24-Hour Urinary Excretion of Calcium in Stone Formers on Normal Diet and with Calcium Supplementation

Value	"t"	P
mg %	1019023	NS (Not Significant)
mg/24 Hours	2.12578	< 0.02
mg/gm Creatinine	0.87887	NS (Not Significant)
mg/m ² BSA	2.709	< 0.01

Table 4: Comparison of Urinary Calcium Excretion on First 24 Hours Urine Sample in Normal Subjects and Stone Formers by Unpaired "t" Test

Subjects	Mean Rise After 2 gm Calcium Loading	Mean Rise After 1 gm Calcium Loading
Normal Subjects (N=20)	10.0 mg	9.1 mg
Stone Formers (N=40)	45.0 mg	34.5 mg

Table 5: Mean Rise in Urinary Calcium Excretion on Normal Diet and Calcium Supplementation

- NH₄CL test was carried out on 4th day, 75 mg/kg body weight group B and 150 mg/kg body weight group A given orally in capsule form at breakfast.
- Hourly urine samples were collected separately over the next seven hours. Each sample was analysed for volume, pH, Calcium (mg %), Inorganic phosphorus (mg %), Creatinine (mg %), Titrable acidity (mg %) and Titrable ammonia (mg %). Observations were tabulated and subjected to statistical evaluation using paired 't' test.

Subjects	1 st Hour	2 nd Hour	3 rd Hour	4 th Hour	5 th Hour	6 th Hour	7 th Hour
Group A (150 mg/kg B. wt.) Normal Subject (N=10)							
Range	11.4-35.0	6.7-29.5	9.2	9.1	4.3-10.3	7.8-20.0	10.-25.0
Mean	23.3	14.9	36.4	16.5	8.6	12.45	15.0
SD±	9.74	7.09	10.40	4.9	2.16	5.2	7.55
Stone Formers (N=20)							
Range	2.5-38.5	2.6-46.5	11.1-43.3	2.6-29.9	7.6-31.0	6.4-37.5	7.4-21.9
Mean	20.97	70.44	20.8	15.85	14.66	16.5	16.0
SD±	1057	11.80	9.56	8.3	7.37	10.2	8.0
Group B (75 mg/kg B > wt.) Normal Subjects (N=10)							
Range	7.3-26.6	9.3-43.2	14.3-42.4	4.5-17.3	9.9-13.7	1.5-21.9	1.66-22.9
Mean	19.9	25.0	20.76	9.7	9.4	10.3	12.0
SD±	7.3	12.3	11.2	4.96	3.3	8.13	8.8
Stone Formers (N=20)							
Range	7.2-33.3	6.4-38.5	6.4-38.5	8.0-20.9	4.0-24.8	6.0-24.3	2.5-32.8
Mean	18.3	15.5	18.41	13.49	12.33	11.33	10.4
SD±	9.19	5.1	9.88	3.88	7.0	6.7	7.54

Table 6: Hourly Urinary Calcium Excretion (In mg) After Loading with 150 mg/kg & 75 mg/kg Body Weight Ammonium Chloride

Twenty four hours urinary calcium excretion in normal subject as well as in stone former was within normal range – 200 mg/24 hours, though it was slightly higher in the former (155.2±87.8 mg/24 hours).

In stone formers (103.8±57.9 mg/24 hours) finding of hypercalciuria is not an important risk. The stone former showed much higher absorption and excretion of calcium as compared with normal subject. The mean rise in 24 hours urinary calcium excretion after 2 gm calcium supplementation was 45.0 mg and 10.8 mg respectively, while on 1 gm calcium 34.5 mg in stone former and 9.5 mg in normal subject this four-fold difference in the absorption of calcium in stone former as compare to normal subject is an important figure brought out by this study and requires further evolution.

Ammonium chloride loading causes increased urinary calcium excretion in stone former as well as in normal subject no significant difference was observed and did not show any evidence of hyperparathyroidism in the subjects investigated.

Subjects	1 st Hour	2 nd Hour	3 rd Hour	4 th Hour	5 th Hour	6 th Hour	7 th Hour
Group A (150 mg/kg B. wt) NH ₄ CL Normal Sub. (N=10)							
Range	50.2-163.0	21.0-137	20.3-65.0	29.2-100.4	8.6-264.0	26.2-100	27.4-120.4
Mean	87.94	63.23	38.3	48.3	34.9	48.0	58.0
SD	47.2	39.0	12.3	27.3	18.65	28.6	33.2
Stone Formers (N=20)							
Range	32.0-182.0	30.0-281	36.5-146	17.1-73.0	19.0-68.5	11.0-109	15.0-105.0
Mean	88.32	83.46	73.9	41.0	35.5	41.8	38.99
SD	52.1	74.26	36.31	14.0	16.7	25.1	24.6
Group B (75 mg/kg B. wt of NH ₄ CL) Normal Sub. N=10							
Range	26.0-121.0	36.6-124	29.0-120	23.0-111	29.0-80	20.0-73.0	19.0-77.0
Mean	74.95	83.3	85.67	52.80	51.3	45.5	47.7
SD±	30.3	32.0	54.0	30.6	19.17	21.33	20.96
Stone Formers (N=20)							
Range	8.0-191.6	18.35-256	20.0-96.7	21.0-104	26.0-118	20.0-156	19.0-152
Mean	94.83	81.3	58.21	51.8	58.5	64.9	71.9
SD±	48.35	73.0	21.7	26.12	27.35	42.7	38.3

Table 7: Hourly Urinary Excretion of Acidity in Normal Subjects and Stone Formers After Loading of Ammonium Chloride

Statistically evaluation of hourly excretion of inorganic phosphorus between normal subject and stone formers was significantly lower in stone former in second and fourth sample and was noted in group A (150 mg/kg body weight) of NH₄CL.

The hourly urinary ammonia excretion after ammonium chloride loading was two times higher in stone former as compared to normal subject in group A, but was not in group B. The difference of ($p < 0.05$) was observed in III urinary samples in group B.

Subjects	1 st Hour	2 nd Hour	3 rd Hour	4 th Hour	5 th Hour	6 th Hour	7 th Hour
Group A (150mg/kg B.W of NH ₄ CL) Normal sub. (N=10)							
Range	4.5-5.0	4.5-5.0	4.5-5.0	5.0-5.5	5.0-5.5	5.0-5.5	5.0-5.5
Mean	4.58	4.66	4.91	5.25	5.25	5.5	5.41
SD±	0.20	0.258	0.20	0.273	0.273	0.316	0.20
Stone Formers (N=20)							
Range	5.0-6.0	5.0-5.0	5.0-6.0	5.0-6.0	5.0-6.0	5.5-6.0	5.5-6.5
Mean	5.53	5.38	5.42	5.42	5.53	5.73	5.76
Group B (75 mg/kg B.W NH ₄ CL) Normal Sub. (N=10)							
Range	4.5-5.5	4.5-5.0	5.0-5.5	5.0-5.5	5.0-5.5	5.5-6.0	5.5-6.0
Mean	5.0	4.83	5.16	5.16	5.25	5.58	5.66
SD±	0.447	0.258	0.258	0.258	0.273	0.204	0.258
Stone Formers (N=20)							
Range	5.0-6.0	5.0-6.0	5.5-6.0	5.5-6.0	5.5-6.0	5.5-6.0	5.5-6.0
Mean	5.54	5.5	5.58	5.58	5.58	5.833	5.875
SD±	0.251	0.301	0.194	0.194	0.325	0.246	0.226

Table 8: Hourly Urinary Excretion of pH After Loading Normal Subjects and Stone Formers with 150 mg/kg B.W. NH₄CL

The ability to bring down the pH of urine up to 4.5 is the criteria on which the adequacy of renal tubular acidification is judged. Failure to achieve this indicates RTA defect.

Group & No. of Case	pH 4.5	pH Between 4.5-5	pH Above 5
Group A (150 mg/kg B.W. of NH ₄ CL) Normal Sub. (N=20) Stone former (N=40)	6 (100.0%) Nil	Nil 6 (46.15%)	Nil 7 (53.8%)
Group B (75 mg/kg mg/kg B.W. of NH ₄ CL) Normal Sub. (N=20) Stone Former (N=40)	3 (50.0%) Nil	3 (50.0%) 3 (25.0%)	Nil 9 (75.0%)

Table 9: Distribution of Cases According to pH of Hourly Urine Samples after Loading with NH₄CL

Parameter	1 st Hour		2 nd Hour		3 rd Hour		4 th Hour		5 th Hour		6 th Hour		7 th Hour	
	t	p	t	p	t	p	t	p	t	p	t	p	t	p
pH	-6.640	<0.001	-6.311	<0.01	-2.921	<0.01	-1.151	NS	-1.848	NS	-1.792	NS	-1.848	NS
Acidity	-0.015	NS	-0.499	NS	2.322	<0.05	0.782	NS	-0.070	NS	0.479	NS	1.405	NS
Ammonia	-2.8028	<0.02	-1.3392	NS	-2.520	<0.05	-1.6297	NS	-2.6722	<0.02	-1.6877	NS	-2.2512	<0.05
Calcium	0.4569	NS	-0.484	NS	-1.143	NS	0.176	NS	-1.948	NS	-0.909	NS	0.05	NS
Phosphorus	0.70	NS	-2.619	<0.02	1.106	NS	2.44	<0.05	0.65	NS	1.281	NS	1.0447	NS

Table 10: Statistical Evaluation of Different Parameters between Normal Subjects & Stone Formers after 150 mg/kg BW of NH₄CL (Group A)

DISCUSSION

Urinary calculus is well known from ancient times and prevalent in several pockets of India including Rajasthan in Udaipur, 56 urinary stone patients per 10000 hospital admissions.^[2] Among stone formers a large number of recurrent stone formers are also present,^[3] many intrinsic and extrinsic factors operate. A sizable group of stone formers classified as “idiopathic stone formers.” In our country renal tubular acidosis and subclinical PTH are two such conditions, which may cause stone episode or recurrence. Short ammonium chloride loading test is good to ascertain HPT status and also good to diagnose renal tubular acidification defect.^[4]

Some nephrocalcinosis patient could not secrete highly acidic urine when challenged with 100 mg/kg body wt. of NH₄CL loading dose and did not induce hypercalcemia in normal subject, but produce profound hypercalcemia in SF

with HPT. In RTA defect reduces reabsorption of calcium result in hypercalciuria.^[1]

Wrong and Davies^[5] suggested a short ammonium chloride test to ascertain the renal acidification status of the kidney; unfortunately has not been used in Indian population. The present study is an effort made in this direction. The mechanism of induction of metabolic acidosis by NH₄CL is very simple; after absorption NH₄CL reaches to liver where ammonia is split off and converted to urea leaving HCL. The disposable of this excess acid [H⁺] is the function of kidney, so as to maintain the subtle acid-base balance in the blood. The kidneys with renal acidification defect show a different degree of failure to excrete the excess load of acid from the body. Normal person within 2 to 3 hours to excrete acidic urine pH below 5, usually 4.5. In the incomplete renal tubular acidosis kidney is unable to acidify urine to a pH lower than 5.0, wherein direct/complete renal tubular acidosis kidney has

very little capacity for acidification with result that alkaline urine is excreted. Turning to renal handling of calcium 50-55% of glomerular filtration of calcium is reabsorbed by proximal convoluted tubules, 20-25% by loop of Henle and 20% by distal convoluted tubules. Parathormone increases the absorption of calcium from intestine, reabsorption from bones and kidney and maintains normal ionic calcium in the blood. In HPT cases reabsorption increases the ionic calcium in the blood. The high ionic calcium causes distal renal acidification defect and leads to hypercalciuria, that is renal tubular acidosis and hyperparathyroidism can concomitantly be tested by acid load with adequate body calcium status. This forms the basis of short ammonium chloride loading test with preload of calcium. This preload of calcium causes profound hypercalciuria in stone patient with HPT, which is absent in normal subject.^[6,7,8,9]

NH₄CL is a gastric irritant, so its proper dose is also standardised.

PYRAH^[10] used 100 mg/kg body wt. of NH₄CL and Danielson et al^[8] administered NH₄CL orally in dose 17.5 gm irrespective of the body wt. in 586 patients [S.F.] and 47 in normal subject. She observed 20% of S.F. having tubular acidification defect. [b] All the N.S. excrete acidic urine pH 5.0 [even up to pH 4.5]. [c] Patients suffering from incomplete renal tubular acidosis excrete acidic urine up to pH 5.0 only. [d] Pt. with renal tubular acidosis type 2nd [Complete RTA] could not acidify urine and excreted alkaline urine [pH more than 7.0]. The pt. with complete and incomplete RTA had more severe stone disease than idiopathic stone formers.

Alken et al^[9] done the same test and found pronounced hypercalciuria in HPT patients. As stated in materials and methods, the 4 days protocol as suggested by Alken et al^[9] was followed with NH₄CL dose 150 mg/kg in group [a] and 75 mg/kg in group [b] instead of 165 mg/kg body wt. was given and addition was the collection of 24 hours urine sample on the days of calcium supplementation. The data of calcium excretion in N.S. and SF presented in Table 2 and 3. The statistical evaluation is given in Table 5 and 6. The 20 normal subjects excreted slightly larger quantity of calcium [155.2+87.8] as compared to S.F. [103.8+57.9 mg/24 hours] with a notable mean rise of calcium level after 1 gm calcium loading. The mean rise 9.1 mg in NS and in SF it was 4 times high [34.5 mg] and 2 gm 45.0 mg in SF and in NS 10.8 mg. This data emphasises the fact that SF have enhanced capacity to absorb calcium from intestine than controls [HPT, poor production of 125-dihydroxycholecalciferol; primary hypercalciuria or renal leak]. In Table 9 and 10 on urinary pH, ammonia and titrable acidity. All NS acidify urine up to pH 4.5 with 150 mg body NH₄CL dose, wherein stone formers 46.2% could acidify the urine between 4.6 to 5.0 and 53.8 and could not acidify urine below pH 5.0. Members from neither of group excreted alkaline urine.

When all data are considered together, it can be concluded that [1] 150 mg/kg body wt. of NH₄CL is sufficient to evaluate the renal acidification defect; [2] 75 mg/kg body wt. NH₄CL is inadequate to evaluate the renal acidification defect; [3] Stone

former absorbed relatively larger dietary calcium than NS. [4] HPT did not appear to be an aetiological factor in any of the stone formers; [5] 53.8% of stone formers appear to have incomplete renal tubular acidification defect. [6] Short ammonium chloride loading test is a reliable adjuvant to evaluate parathyroid and renal acidification defect.

SUMMARY AND CONCLUSION

1. Incomplete R.T.A. defect was present in 53.8% of stone formers investigated.
2. Complete R.T.A. defect was not present in any of the stone formers.
3. Hyperparathyroidism was not present in any of the subjects. It denotes HPT cases are not present in this region [Udaipur – Rajasthan].
4. Short ammonium chloride loading test is a reliable tool to evaluate the presence of Hyperparathyroidism and Renal Tubular Acidification Defect.
5. For a reliable test, ammonium chloride has to be given in the dose of 150 mg/kg body weight.
6. Hypercalciuria is not a feature amongst the local the local population in both stone formers and in normal subjects.
7. The stone formers show higher absorption and excretion of calcium on oral supplementation of calcium as compared to normal subjects.

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