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Vitamin D status in primary hyperparathyroidism in India

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Summary

OBJECTIVES Primary hyperparathyroidism is a syndrome with variable clinical expression, presenting as asymptomatic hypercalcaemia in Western countries and with predominant bone disease in developing countries. Vitamin D deficiency has been implicated as the cause of bone disease. There is a paucity of information on the vitamin D ($25\text{-}OHD_3$) status of patients with primary hyperparathyroidism presenting with bone disease. The present study aims to evaluate the vitamin D status in patients with primary hyperparathyroidism and to correlate it with the bone disease.

DESIGN Twenty consecutive patients with primary hyperparathyroidism admitted to the endocrinology and metabolism wards of the All India Institute of Medical Sciences were analysed to assess their clinical, radiological and biochemical features, as well as parathyroid hormone (mid-molecular, PTH-MM) and $25\text{-}OHD_3$ levels.

MEASUREMENTS PTH-MM levels and $25\text{-}OHD_3$ levels were measured using RIA kits.

RESULTS Bone disease (osteitis fibrosa cystica) was the mode of presentation in 90%. Radiologically, sub-periosteal resorption was present in 90% of the total group of patients, brown tumours in 60%, and pathological fractures in 40%. Renal stones and/or nephrocalcinosis was present in 50% of patients. Mean serum calcium, phosphate and alkaline phosphatase concentrations (mean of 3 days values) were $2{\cdot}72 \pm 0{\cdot}24$ mmol/l; $1{\cdot}01 \pm 0{\cdot}28$ mmol/l and 425 ± 249 IU/l respectively. The 24-hour (mean of 3 days values) urine calcium and phosphate excretions were $8{\cdot}0 \pm 4{\cdot}2$ mmol and $19{\cdot}0 \pm 13$ mmol. Only 50% of the patients had hypercalcaemia (> 2·7 mmol/l). However, 90% of the whole group of patients had hypercalciuria. The mean serum creatinine concentration of patients with hypercalcaemia was 108 ± 38 μmol/l and of those with normocalcaemia 89 ± 33 μmol/l. The mean serum PTH-MM was 438 ± 350 pmol/l (the detection limit for the kit was 34 pmol/l). Ultrasound examination detected adenomas in 72% of the cases and computerized tomography of the neck localized adenomas in 71% of the cases. The median weight of the adenoma was 4·6 g (range 0·125–25 g). Two patients had coexistent hyperplasia of the other parathyroid glands and two had recurrent adenomas. $25\text{-}OHD_3$ levels were assessed in all 20 patients under fasting conditions. The mean value of $25\text{-}OHD_3$ observed ($8{\cdot}4 \pm 5{\cdot}1$ μg/l) was comparable to the mean value measured in 14 healthy age and sex matched controls ($8{\cdot}3 \pm 2{\cdot}5$ μg/l).

CONCLUSION Patients with primary hyperparathyroidism in India presented with bone and renal diseases; half were normocalcaemic. All the patients had hypercalcuria despite the bone disease. The PTH-MM levels were increased and $25\text{-}OHD_3$ levels were low. The predominant bone disease is probably due to prolonged primary hyperparathyroidism coexisting with low calcium intake and/or $25\text{-}OHD_3$ deficiency. The mean weight of the adenoma was higher than that reported for patients in the Western literature.

Primary hyperparathyroidism is a syndrome with variable clinical presentation. Albright's original studies (Albright *et al.*, 1934; Albright & Reifenstein, 1948) characterized primary hyperparathyroidism as a disease of 'bones and stones'. In some patients, primary hyperparathyroidism was complicated by nephrolithiasis, nephrocalcinosis and renal insufficiency. In others, a distinctive bone disease predominated. With the advent of multichannel serum autoanalysers a new clinical phenotype of primary hyperparathyroidism is recognized (Lafferty, 1981). Thus the phenotypic expression of the disease varies in different parts of the world. In developing countries such as India it still manifests predominantly as a bone disease and/or renal stone disease. In developed countries where all hospitalized patients undergo routine serum calcium estimation by autoanalyser, the majority of hyperparathyroidism patients are detected by the finding of hypercalcaemia (Heath *et al.*, 1980; Mundy *et al.*, 1980; Hellström & Ivemark, 1962; McGeown & Morrison, 1959). The reason why this disease predominantly presents with bone disease in developing countries is not known. Vitamin D deficiency has been implicated in this regard (Ahuja, 1974; Kapur *et al.*, 1985;

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Insogna *et al.*, 1985; Wills *et al.*, 1969). However there is a paucity of information on the vitamin D status of patients with primary hyperparathyroidism who predominantly present with bone disease. In view of this we carefully characterized 20 consecutive cases of primary hyperparathyroidism, documenting clinical, radiological, biochemical and histopathological factors, and relating these to vitamin D status.

Materials and methods

Twenty consecutive cases of primary hyperparathyroidism attending the endocrine clinic between January 1989 and June 1993 were studied. All were admitted to the endocrine and metabolic ward for investigations. Basal blood samples were collected on three consecutive days for estimation of serum calcium, phosphate, alkaline phosphatase (SAP), creatinine, and albumin concentrations. Simultaneously, 10 ml of blood was collected in chilled test-tubes kept on ice, for serum parathyroid hormone mid-molecule (PTH-MM) and 25-hydroxyvitamin-D_3 (25-OHD_3) measurements. Serum was separated and stored at −20°C for subsequent estimations. The patients were asked to collect 24-hour urine specimens in calcium-free containers for determination of urinary calcium, phosphate and creatinine. Serum calcium was estimated by the method of Wilkinson (1957), serum phosphate by the method of Gomori *et al.* (1942), and serum alkaline phophatase by the method of McComb *et al.* (1979). The normal laboratory range for total serum calcium was 2·25–2·7 mmol/l, serum phosphate 0·80–1·50 mmol/l and serum alkaline phosphatase 21–92 IU/l in adults and 64–277 IU/l in children. From these values the calcium/creatinine ratio (Ca/Cr ratio), and phosphate excretion index (PEI) were calculated (Nordin & Fraser, 1960). The serum samples were analysed for PTH-MM and 25OHD_3 using RIA kits purchased from Incstar Corporation (Minnesota, USA) 51065 for PTH-MM and kit 58100 for 25-OHD_3. The detection limit in the PTH-MM assay was 34 pmol/l. The quality control sera provided with the kit gave values of 85 pmol/l for control serum 1 and 155 pmol/l for control serum 2. (The quality control specifications in the kit for control serum 1 was 78·6 pmol/l (range 64·9–92·3) and for control serum 2 was 126 pmol/l (range 102–149)). The 25-OHD_3 assay involved an extraction step with acetonitrile followed by RIA. The detection limit in the vitamin D_3 assay was 2·9 μg/l.

An abbreviated skeletal survey (radiographs of lateral skull, PA chest, abdomen for KUB region, AP pelvis, both hands and lateral dorsolumbar spine) was done in all the patients. Ultrasound of the neck and subsequently computed tomography of the neck were done in all patients for localization of the parathyroid glands. On completing the investigations and diagnosis, patients were subjected to neck exploration by the endocrine surgeon of the AIIMS, New Delhi.

Results

Clinical

Clinical profiles of the 20 patients of primary hyperparathyroidism studied are shown in Table 1. The M : F ratio was 1 : 2 with a mean age of 38 ± 15 years (range 14–62 years).

Ninety per cent of patients presented with a history of bone pains and 40% with bone fractures. Ten per cent of patients had coexisting clinical features of rickets in the form of genu valgum and expansion of the wrists. Proximal muscle weakness was found in 70% of patients. None of the patients presented with clinical signs and/or symptoms of hypercalcaemia or uraemia. Pancreatitis was the mode of presentation in one patient and another patient developed pancreatitis during the course of the hospital stay. Non-specific fatigueability was found in 100% of patients and hypertension in 40%. None of the patients presented with renal colic.

Radiological

The main radiological findings in the 20 patients are summarized in Table 2. The radiological picture of osteitis fibrosa cystica was found in the vast majority (95% of patients with 'pepper-pot' skull; 90% sub-periosteal erosions and 60% with brown tumours). Pathological fracture was found in 40% of the patients. Radiological evidence of renal stone disease was present in 60% of the patients. Of these, one-third had obstructive uropathy and the remaining two-thirds nephrocalcinosis.

Table 1 Clinical profile

Symptoms	Patients affected (%)
Bone pains	90
Bone deformities	10
Fractures	40
Abdominal pain	30
Pancreatitis	10
Fatigueability	100
Proximal muscle weakness	70
Hypertension	40

Table 2 Summary of radiological (bone and renal) profile

Parameter	No. of cases	(%)
Bone deformity	2/20	10
Changes in skull	19/20	95
Sub-periosteal resorption	18/20	90
Brown tumours	12/20	60
Pathological fractures	8/20	40
Renal disease	10/20	50
Renal stones	5/10	50
Ureteric stones	3/10	30
Nephrocalcinosis	6/10	60

Biochemical profile

On the basis of the serum calcium concentration, the 20 patients were placed in two groups (Table 3a) namely, normocalcaemic and hypercalcaemic primary hyperparathyroidism. Interestingly, roughly 50% of the patients were normalcaemic (2·54 ± 0·17 mmol/l) and the remaining 50% were hypercalcaemic (2·9 ± 0·14 mmol/l). The difference in the mean calcium values between the two groups was statistically significant ($P < 0·05$). The mean serum creatinine concentration in patients with hypercalcaemia was 108 ± 38 μmol/l and in those with normocalcaemia was 89 ± 33 μmol/l.

The mean serum phosphate concentration observed (Table 3b) in the whole group of primary hyperparathyroidism patients was 1·01 ± 0·28 mmol/l. This was not significantly different from the mean value measured in normal individuals studied in our laboratory. Even in the presence of phosphaturia comparable to values reported from hyperparathyroid patients from Western countries, the serum phosphate levels were normal in the majority. Only 25% of the patients had low serum phosphate levels.

The mean SAP was high in all but one of the present group of primary hyperparathyroid patients (mean 425 ± 249 IU) (laboratory normal 21–92 IU). Interestingly, the patient who had normal SAP had no radiological bone disease (Table 3b; patient 14). There was significant positive correlation between serum calcium and serum alkaline phosphatase activity ($r = 0·47$; $P < 0·05$).

Serum PTH-MM measurement showed a mean value of 438 ± 350 pmol/l in this group. Three of the 20 patients had PTH within the normal range. There were positive correlations between both serum and urinary Ca/Cr ratio and the weight of the resected adenomas ($r = 0·51$, $P < 0·05$).

The mean 25-OHD_3 measured in this series of patients was 8·39 ± 5·09 μg/l. The mean value of vitamin D_3 measured in ten normal age and sex matched controls from Delhi was 8·29 ± 2·5 μg/l. However, the difference was not statistically significant and may be due to the small number of subjects studied. A strong positive correlation

Table 3a Hyperparathyroidism: biochemical and hormonal profile

Variable	Hypercalcaemic group	Normocalcaemic group	Normal range
Age (years)	38.22 ± 16.67	35.8 ± 13.8	
S. Ca (mmol/l)	2·9 ± 0·14	2·54 ± 0·17	2·25–2·74
S. Pho (mmol/l)	1·04 ± 0·3	0·98 ± 0·27	0·8–1·5
SAP (IU)	513 ± 244	337 ± 234	21–92
S. Cr (μmol/day)	108 ± 38	88·9 ± 32·8	53·04–141·44
U. Cr (nmol/day)	4106 ± 1241	6546 ± 2385	
U. Ca (mmol/day)	6·8 ± 2·7	9·2 ± 5·1	
U. Pho (mmol/day)	12·6 ± 4·8	25·3 ± 15·6	
Total urine (l/day)	1·439 ± 0·646	1·960 ± 0·77	
Ca/Cr ratio	1·69 ± 0·61	1·46 ± 0·65	0·3
PEI	0·285 ± 0·223	0·31 ± 0·24	0·09
PTH-MM (pmol/l)	638 ± 398	260 ± 176	29–85
25-OHD_3 (μg/l)	8·74 ± 5·27	8·05 ± 5·18	14·2–40
S. Alb (g/l)	38 ± 3	40·2 ± 4·9	
Wt. of gland (g)	10·75 ± 10·5	3·90 ± 3·95	
Cr/cl (ml/min)	29 ± 12	54 ± 20	

Values are mean ± SD.
Ca, Calcium; Pho, phosphate; AP, alkaline phosphatase; Cr, creatinine; PEI, phosphate excretion index; PTH (MM), parathormone mid-molecule; 25-OHD_3, 25-hydroxyvitamin D_3.

Table 3b Hyperparathyroidism: biochemical and hormonal profile

S. No.	Age	Name	Sex	S.Ca (mmol/l)	S.Phos (mmol/l)	SAP (IU)	S.Cr (μmol/l)	U.Cr (μmol/day)	U.Ca (mmol/day)	U.Phos (mmol/day)	Total (l/day)	Ca/Cr Ratio	PEI	PTH-MM (pmol/l)	25-OHD_3 (μg/l)	S.Alb (g/l)	Wt. of gland (g)	Cr/cl (ml/min)
1	55	C	M	3·12	1·23	973	159	5039	4·3	15·1	1·650	0·86	0·378	110	5·25	38	4·000	22
2	50	B	F	2·7	1·72	234	124	4022	6·3	10·3	1·235	1·58	−0·06	43	19·25	42	–	23
3	35	S	F	2·52	0·97	398	97	5331	9·18	22·5	1·665	1·72	−0·40	227	18·00	39	7·700	38
4	35	B	F	2·82	0·88	710	168	5039	11·0	8·1	1·565	2·18	−0·28	1000	5·04	39	25·000	21
5	27	R	F	2·59	0·73	738	62	3792	9·3	19·4	1·400	2·45	−0·40	560	4·20	39	1·800	43
6	25	W	M	2·72	1·39	178	71	3925	9·5	12·5	2·165	2·42	0·155	46	12·43	28	–	39
7	15	S	F	2·57	1·45	334	88	6303	8·7	12·1	1·334	1·39	0·108	35	8·02	41	4·700	50
8	44	BS	M	2·10	1·01	131	97	7010	5·6	41·1	2·432	0·80	−0·55	254	14·07	42	0·700	50
9	55	BL	M	2·99	0·70	407	103	5829	10·6	17·2	1·434	1·82	−0.065	814	7·56	38	–	39
10	46	AN	M	2·64	1·00	317	162	11554	22·6	61·4	3·750	1·95	−0.843	–	3·88	44	–	50
11	37	S	F	2·74	0·86	267	77	2829	8·4	17·6	1·567	2·96	−0.53	1000	12·81	35	–	26
12	14	MK	M	3·03	0·72	412	73	5428	7·8	6·3	2·600	1·44	−0·087	–	7·77	40	4·500	51
13	55	BK	F	3·01	1·08	249	141	2679	5·7	15·9	1·540	2·13	−0.766	708	14·59	40	7·000	13
14	62	CP	M	2·52	0·82	65	80	8310	9·4	19·0	1·167	1·13	−0.196	113	3·99	39	11·000	73
15	28	SD	F	2·88	0·97	520	103	2802	4·3	5·9	1·467	1·52	−0·209	–	2·94	32	22·000	19
16	35	CK	F	2·67	0·61	375	35	5163	6·3	22·0	1·653	1·23	−0·210	326	4·57	42	5·000	101
17	15	SG	F	2·82	1·00	710	62	2776	3·0	11·8	0·784	1·10	−0·247	426	3·57	40	2·200	31
18	38	S	F	2·85	1·23	646	71	4623	6·1	18·4	1·866	1·31	−0·227	1000	8·60	35	–	45
19	45	B	F	2·48	0·90	128	97	8522	7·7	31·4	2·433	0·90	−0·356	417	8·40	47	0·125	61
20	24	HB	F	2·58	0·87	710	100	5552	3·6	12·1	1·600	0·65	−0·228	363	2·94	41	0·200	34
Mean	37			2·72	1·01	425	99	5326	8·0	19·0	1·765	1·58	0·315	437·8	8·39	39·1	6·85	41
SD	14·4			0·24	0·28	249	36	2234	4·1	13·0	0·649	0·62	0·218	349·9	5·09	4·2	7·71	21

Values are mean ± SD.

Ca, Calcium; Pho, phosphate; AP, alkaline phosphatase; Cr, creatinine; PEI, phosphate excretion index; PTH (MM), parathormone mid-molecule; 25-OHD_3, 25-hydroxyvitamin D_3.

was observed between serum 25-OHD_3 concentration and PEI ($r = 0{\cdot}62$; $P < 0{\cdot}05$).

Ninety per cent of patients had hypercalciuria as shown by 24-hour urinary calcium excretion or Ca/Cr ratio. There was a positive correlation between PTH-MM and Ca/Cr ratio ($r = 0{\cdot}6$; $P < 0{\cdot}05$) indicating progressively increasing hypercalciuria with increasing PTH levels. An interesting observation was the positive correlation between Ca/Cr ratio and the weight of the parathyroid adenoma resected ($r = 0{\cdot}5$; $P < 0{\cdot}05$) indicating a relation between hypercalciuria, degree of hyperparathyroidism (as evidenced by PTH-MM levels) and weight of the parathyroid adenoma. This again was confirmed by a positive correlation between the PTH-MM levels and weights of the resected adenoma ($r = 0{\cdot}53$; $P < 0{\cdot}05$). There was a positive correlation between the weight of the adenoma and the urinary Ca/Cr ratio ($r = 0{\cdot}5$; $P < 0{\cdot}05$) and a negative correlation between weight of the gland and urinary phosphate excretion ($r = 0{\cdot}44$; $P < 0{\cdot}05$). These correlations demonstrate the internal consistency of the data presented.

Ultrasound and computed tomography

The ultrasound and computed tomographic findings of the individual patients are given in Table 4. Ultrasound examination detected adenomas in 72% of the cases and CT scan localized adenomas in 71% of cases.

Histopathology

Seventeen of the 20 patients underwent neck exploration. Fourteen of the 17 glands were weighed at surgery. The median adenoma weight was 4·6 g (range 0·125–25 g) (Table 5). Chief cell adenoma was found in 70% of patients and mixed cell adenoma in 12%. Eighteen per cent of the patients had hyperplasia of all four glands. Twelve per cent of the patients had recurrent adenomas.

Discussion

In the present study, the mean age at presentation of hyperparathyroidism was twenty years younger than the mean age described in reports from developed countries, where the average age at presentation is in the sixth or seventh decades of life (Heath, 1989). In our series 45% presented with pure skeletal disease, 5% with renal stone disease and 50% with both skeletal and renal stone disease. We have not screened for asymptomatic primary hyperparathyroidism and hence have no data on the prevalence of this entity in our local population. In developed countries (Heath, 1991) over the last 25 years, the clinical spectrum of primary hyperparathyroidism has changed. The previously reported prevalence of 57% renal stone disease and 35% radiological bone disease have fallen to less than 5% for both modes of presentation in recent years (Heath, 1991). With the introduction of routine calcium estimations by autoanalyser in all patients attending hospitals, the incidental finding of hypercalcaemia has become the main way in which patients of hyperparathyroidism are detected in developed countries (Heath, 1991). As a result, a presentation with bone disease is rarely seen in Western clinical practice. However, clinical presentation of the disease in India continues to be the same as that classically described by Albright and Reifenstein (1948) half a century ago (Albright *et al.*, 1934). Hypercalcaemic symptoms were conspicuous by their absence in our group of patients when compared to their presence in 99% of patients with primary hyperparathyroidism in Western countries (Heath, 1989).

In the present group of patients only half had hypercalcaemia (serum calcium $2{\cdot}9 \pm 0{\cdot}14$ mmol/l) and the remaining were normocalcaemic. This is in contrast to Western experience where the vast majority of patients with hyperparathyroidism have hypercalcaemia at presentation (Heath, 1991). There are only very few and exceptional reports of normocalcaemic hyperparathyroidism with bone and stone disease, from Western countries (Wills *et al.*, 1969; Hodkins, 1963; Frame *et al.*, 1970). In India all patients with primary hyperparathyroidism had bone disease (Kapur *et al.*, 1985; Sridhar *et al.*, 1973; Shrikande *et al.*, 1980; Gupta, 1990) and 53% had associated renal stone disease. The cause of the high prevalence of normocalcaemic primary hyperparathyroidism in our series is probably due to concomitant 25-OHD_3 deficiency and/or dietary calcium deficiency/malabsorption. The low levels of 25-OHD_3 levels measured in all the patients in the present series tend to support this possibility.

Ninety per cent of our patients had hypercalciuria. Even patients with clinical features of rickets had hypercalciuria. There was a positive correlation between PTH-MM and Ca/Cr ratio. Friedman and Gesek (1993) have shown in in-vitro studies using mouse distal convoluted tubule (DCT) cells, that 1,25-dihydroxy D_3 accelerates PTH dependent calcium reabsorption in distal convoluted tubules. The relative deficiency of 25-OHD_3 in our patients when coupled with high PTH may be causing severe bone disease as a result of poor calcium resorption by the DCT and consequent negative calcium balance. Also the severely affected bones may not be a good source of calcium by resorption. The 25-OHD_3 deficiency related poor calcium absorption from the gut may worsen the negative calcium balance in our

Table 4 Hyperparathyroidism: imaging results

No.	Age	Sex	Ultrasound of abdomen	Ultrasound of neck	Computed tomography of neck
1	55	M	Renal stones (r and l)	L. inf. pole adenoma	L. inf. pole adenoma
2	50	F	Nephrocalcinosis	Normal	R. inf. pole adenoma
3	35	F	Small r. kidney L. hydronephrosis L. renal stone	? R. inf. pole adenoma	R. inf. pole adenoma
4	34	F	R. renal stone	L. inf. pole adenoma	–
5	27	F	–	L. inf. pole adenoma	–
6	25	M	Pancreatitis nephrocalcinosis right side	No mass seen	No mass seen
7	15	F	Normal	No mass seen	? R. inf. pole adenoma
8	44	M	Bilateral nephrocalcinosis	–	R. sup. adenoma
9	55	M	–	L. sup. adenoma	L. sup. adenoma
10	46	M	Normal	–	R. sup. adenoma
11	37	F	B/L nephrocalcinosis	R. sup. adenoma	L. sup. adenoma
12	14	M	Pancreatitis	R. sup. adenoma	R. sup. adenoma
13	55	F	–	L. sup adenoma	R. inf. adenoma
14	62	M	R. renal stone	L. inf. adenoma	L. inf. adenoma
15	28	F	Nephrocalcinosis	L. inf. adenoma	L. inf. adenoma
16	35	F	Normal	No mass seen	No mass seen
17	15	F	Normal	L. sup. adenoma	–
18	38	F	Nephrocalcinosis	R. inf. adenoma	R. inf. adenoma
19	45	F	? L. renal stone	R. sup. adenoma	? R. sup. adenoma
20	24	F	Normal	L. inf. adenoma	No definite adenoma

patients. Furthermore, the high phosphate and phytate content, associated with dietary calcium deficiency, in the Indian diet may contribute to major negative calcium balance in our patients with hyperparathyroidism in India. This may explain the fact that half of our patients are normocalcaemic.

Interestingly, the mean serum phosphate was normal in the present group of patients and only 25% of them had hypophosphataemia. This contrasts with almost invariable hypophosphataemia in patients with hyperparathyroidism reported from the West (Gorden & Roof 1968; Bilezikian *et al.*, 1991). All the patients in the present group had demonstrably raised PEI. The markedly increased phosphate intake in Indian diet may explain the normophosphataemia in the majority of our patients, even in the presence of increased PEI, apparently caused by hyperparathyroidism.

The 25-OHD_3 levels measured in the present group of patients showed a mean of $8{\cdot}39 \pm 5{\cdot}09\ \mu g/l$ (normal range provided with the USA-manufactured kit $14{\cdot}2–40\ \mu g/l$). Two out of the 14 normal Indian controls had values below $5\ \mu g/l$ compared to 7 out of 20 showing 25-OHD_3 below $5\ \mu g/l$ in the patients. There are no similar data available from a developing country for comparison. However, in a study by Silverberg *et al.* (1989; 1990) on patients with primary hyperparathyroidism without bone disease, the mean 25-OHD_3 measured was $20 \pm 2\ \mu g/l$ (normal $9–52\ \mu g/l$). Vitamin D deficiency has been shown to reduce hyperabsorption of calcium from the gut in hyperparathyroidism (Insogna *et al.*, 1985; Lumb & Stanburg 1974; Dent *et al.*, 1961). When primary hyperparathyroidism coexists with low vitamin D status there could be accelerated bone changes resulting in overt bone disease. The presence of bone disease in the majority of our patients, coupled with the observation of normocalcaemia in half of them, strongly

Table 5 Hyperparathyroidism: pathological features

No.	Age	Sex	Wt. of gland (g)	Biopsy report
1	55	M	4·0	Chief cell adenoma
2	50	F	–	Clear and focal acidophil adenoma
3	35	F	7·7	Chief cell adenoma
4	35	F	25·0	Clear cell adenoma
5	27	F	1·8	Chief cell adenoma
6	25	M	Patient died due to pancreatitis	
7	15	F	4·7	Hyperplasia*
8	44	M	0·7	Chief cell adenoma
9	55	M	–	Chief cell adenoma
10	46	M	Patient refused surgery	
11	37	F	–	Chief cell adenoma
12	14	M	4·5	Chief cell adenoma
13	55	F	7·0	Chief cell adenoma
14	62	M	11·0	Clear cell adenoma
15	28	F	22·0	Oxyphil and foci of chief cells
16	35	F	5·0	Clear cell adenoma
17	15	F	2·2	Chief cell adenoma
18	38	F	Not yet operated	
19	48	F	0·125	Hyperplasia
20	24	F	0·2	Nodular hyperplasia

*Hyperplasia of right inferior (4·7 g), left inferior and superior glands (0·7 and 0·2 g). Right superior gland was left *in situ*.

suggests that coexisting vitamin D deficiency and relative calcium deficiency may be playing an important contributory role in the pathogenesis of the severe osteitis fibrosa cystica in our group of hyperparathyroid patients. In this regard it is relevant to emphasize that even the normal adults ($n = 14$) studied had relatively low levels of 25-OHD_3 ($8{\cdot}29 \pm 2{\cdot}5\ \mu g/l$), compared with Western populations. These findings suggest that sub-clinical 25-OHD_3 deficiency may well be widely prevalent in tropical countries such as India despite plentiful sunlight. However, large population based studies on vitamin D levels are required to scientifically confirm this possibility.

There are reports of rickets coexisting with primary hyperparathyroidism from both India (Ahuja, 1974; Kapur *et al.*, 1985; Sridhar *et al.*, 1973) and developed countries (Lomnitz *et al.*, 1966; Rapaport, 1986). In the present group of 20 patients there were three who were less than 15 years. Interestingly two of the three had radiological abnormalities suggestive of rickets. These observations when coupled with the low vitamin D levels measured suggest that in India, parathyroid adenomas occur in a clinical setting of wide prevalence of vitamin D deficiency, and consequent negative calcium balance. Indeed some of them may well have coexisting hyperplasia of the remaining parathyroid glands. Indeed, in one of our patients (no. 7, Table 5) neck exploration revealed a parathyroid adenoma (right inferior) which weighed 4·7 g; furthermore, the left superior and inferior glands of this patient were found to be enlarged and were resected (0·7 and 0·2 g in weight respectively). 25-OHD_3 levels were low in this patient ($8{\cdot}02\ \mu g/l$). Hyperparathyroidism induces excessive bone resorption and interferes with normal mineralization of growing bone of children (Lomnitz *et al.*, 1966; Rapaport *et al.*, 1986; Girad *et al.*, 1982).

A positive correlation was observed between the weight of the adenoma and the SAP levels in the current group of patients. These findings suggest increased severity of bone disease with increasing weight of the parathyroid gland adenoma. Norris (1947) reported a median gland weight of 7 g in a study of 322 patients. However, recent reports (Rasbach *et al.*, 1984; Wang & Rieder, 1978) refer to parathyroid microadenomas whose weight ranges from 13 to 57 mg. The trend of declining weights of resected glands in the West may be due to the fact that in more and more cases the disease is being diagnosed in the early asymptomatic phase due to calcium screening. In our series the median weight was 4·6 g (range 0·125–25 g). With low vitamin D levels, and relatively low calcium levels, the impressively large size of adenomas resected from our patients is consistent with the known determinants of parathyroid cell proliferation. Thus several observations made during the present study seem to link vitamin D deficiency and predominant bone disease in the present group of hyperparathyroid patients.

Summary

Primary hyperparathyroidism seen in this study from India manifested with bone disease and renal disease.

Only half the patients had hypercalcaemia and the rest were normocalcaemic.

All patients had hypercalciuria despite severe bone disease.

The serum PTH-MM levels were increased and 25-OH vitamin D_3 levels were low in Indian patients with primary hyperparathyroidism.

Predominant bone disease is probably due to prolonged primary hyperparathyroidism coexisting with low calcium intake and/or vitamin D deficiency in these patients.

The mean adenoma weight was higher in our group than those quoted in the recent Western literature. One patient had coexistent hyperplasia of the other parathyroid glands and two had recurrent adenomas.

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Original Article

Vitamin D status in primary hyperparathyroidism in 1990 and thence – Emergence of normocalcaemic presentation and diagnostic challenges – Utility of parathyroid function index

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Abstract

Background: 25-hydroxyvitamin D (25OHD) levels much influence parathyroid hormone levels and bone disease in primary hyperparathyroidism (PHPT). With the emergence of the normocalcaemic PHPT (NCPHPT), repletion of the 25OHD level to rule out secondary hyperparathyroidism (SHPT) is essential. This may delay the diagnosis of PHPT, and a diagnostic tool like parathyroid function index (PF index) may help in the early diagnosis.

Methods: The biochemical and hormonal profiles of 52 patients with PHPT were analysed and compared with first description in 1990. Patients were grouped based on symptoms and albumin-corrected serum calcium levels. Those with normocalcaemia were subgrouped into those with and without 25OHD deficiency. Data were extracted from the hospital's electronic medical records to find subjects with SHPT and normal controls and calcium-to-phosphate ratio (C/P ratio) and the PF index were calculated. Receiver operating characteristic curves to decide the cut-off values that help in identifying PF index and C/P ratio between various subgroups.

Results: Sixty-two per cent (32/52) were asymptomatic, 40% (21/52) normocalcaemic, amongst which 48% (10/21) had normal 25OHD levels. Across all categories, the PF index was more sensitive, specific and superior compared to the C/P ratio in the diagnosis of PHPT ($P = 0{\cdot}02$), NCPHPT ($P = 0{\cdot}03$) or SHPT ($P = 0{\cdot}0001$). PF index (>25·8) was more sensitive (90%), specific (96.51%), compared to C/P ratio (>0·211) (P = 0·04) in differentiating NCPHPT from SHPT.

Conclusions: The prevalence of asymptomatic PHPT and NCPHPT is on the rise. PF index helps distinguish NCPHPT from SHPT minimising the time required for confirming the diagnosis post-25OHD repletion.

Keywords: 25-hydroxyvitamin D, asymptomatic primary hyperparathyroidism, calcium-to-phosphate ratio, normocalcaemic primary hyperparathyroidism, parathyroid function index, primary hyperparathyroidism, secondary hyperparathyroidism

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INTRODUCTION

Primary hyperparathyroidism (PHPT) is the third most common endocrine disorder with variable clinical presentation in an outpatient setting. Fuller Albert (1948)[1] described the disease, as one with bone, stones, abdominal moans and groans. During the earlier decades from 1930 to 1970, PHPT was an asymptomatic disorder with overt skeletal and renal complications. Since the early 1970s, PHPT was increasingly reported as an asymptomatic disease.[2] In early 1953[3] and later, other workers[4,5] described a new phenotype of PHPT biochemically characterised by normal calcium levels, the NCPHPT. Widespread testing and improved sensitivity of biochemical tests have led to increasing recognition of PHPT in recent times, both in normocalcaemic and asymptomatic patients. Most of the cases identified while screening for low bone mass and osteoporosis in the early 21st century turned out as NCPHPT.[6] Earlier attempts to define PHPT had not considered the 25OHD levels as part of the definition.

Occurrence of 25OHD deficiency in PHPT was first documented in 1971.[7] In 1990, an observational study from India involving PHPT patients found that nearly half of PHPT patients were normocalcaemic at presentation. The same study also documented low 25OHD levels in both normocalcaemic and hypercalcaemic patients with PHPT.[8] Since then, there is increasing recognition of the role of 25OHD on PTH levels as a potential confounding factor in the diagnosis of PHPT, particularly NCPHPT. This has led to a consensus towards defining the entity (NCPHPT) at the fourth international workshop on the management of asymptomatic PHPT in 2014.[9] Currently, NCPHPT is a diagnosis of exclusion with the following criteria: (a) raised PTH levels with normal albumin-adjusted (corrected) serum calcium (ACSC) and ionised calcium levels; (b) 25OHD levels >30 ng/mL; (c) adequate dietary calcium intake; (d) creatinine clearance >60 mL/Min; (e) ruling out a secondary cause of PTH elevation and (f) not on medications including loop diuretics, thiazides, lithium, bisphosphonates or denosumab.[9,10]

Published literature on PHPT dating back to years before this recent consensus encompass a spectrum of PHPT ranging from normocalcaemia to hypercalcaemia with coexistent 25OHD deficiency when measured. In such a situation, wherein PHPT may coexist with low Vitamin D status, phosphate values could help in distinguishing 25OHD deficiency and PHPT, particularly the NCPHPT, as both these disorders may present with normocalcaemia and high PTH levels. Whereas PHPT is classically associated with relatively higher serum calcium but lower phosphate concentration, it is not so with 25OHD deficiency. Given this, we explored the calcium-to-phosphorus ratio (C/P ratio) as a tool to find PHPT more reliably.[11] While the C/P ratio remains an attractive and simple tool, PTH levels that define PHPT and NCPHPT are not accounted for in their measurement limiting their potential. Hence, a new index, namely PF index, was developed using calcium, phosphorus and PTH together to magnify the biochemical differences between disorders both characterised by raised PTH, thereby helping in the diagnosis of PHPT, particularly NCPHPT despite coexistent low 25OHD status.[12]

MATERIAL AND METHODS

The study was initiated after obtaining Institutional Ethics Committee approval (SWH_IEC_11/Dr.HCV/Ju 6, l2021, dated July 14, 2021).

We included patients, presenting to the department of endocrinology and metabolism, who underwent surgery after being evaluated for PHPT from 2014 to 2021 in the study. We profiled the biochemical, hormonal, functional, structural imaging and bone mineral density (BMD) of PHPT patients with radiologically proven adenomas who then underwent parathyroidectomy and carefully characterised them as symptomatic versus asymptomatic PHPT and hypercalcaemic versus normocalcaemic variants of PHPT. We developed a new cut-off ratio for parathyroid index (PF index) after including data from a large number of normal patients with and without adequate 25OHD levels as controls and used it to redefine the diagnosis of PHPT and NCPHPT in our cohort. We also compared our first documented biochemical profile levels in patients with PHPT in 1990 with the present data of 2021 to look at the pattern of change in the past three decades with improving the Vitamin D and calcium status of the population.

We estimated serum albumin, creatinine, calcium, phosphorus, 25OHD and intact PTH from fasting blood samples collected without using a tourniquet. Urine calcium: creatinine ratio was also evaluated on a fasting sample. We evaluated the biochemical parameters serum calcium, phosphorous, alkaline phosphatase – SAP, creatinine and albumin by UniCel DxE860 (i) (Beckman Coulter, Inc., USA). We used UniCel Dxl 600 Access Immunoassay System (Beckman Coulter, Inc., USA) autoanalyser to analyse 25OHD and intact parathormone. The normal range of biochemical parameters in the serum was as follows: calcium 8.9–10.5 mg/DL, phosphorous 2.5–4.6 mg/dL, SAP 32–126 IU/L, albumin 3.5–5.0 g/dL, creatinine 0.61–1.2 mg/dL, 25OHD >30 ng/mL and PTH 12–88 pg/mL. We corrected serum calcium for albumin.

We used albumin corrected serum calcium (ACSC) for the analysis of data. We calculated the C/P ratio and PF index from the biochemical parameters obtained. We calculated the C/P ratio by dividing serum calcium and serum phosphorous in mM (Ca/P). We calculated the PF index by multiplying PTH (mM) by ACSC (mM) and divided by serum phosphorous (mM) (PTH × ACSC/serum phosphorous).[11,12]

We recorded BMD of the spine and hip. We excluded patients with creatinine clearance <30 mL/Min and on medications including corticosteroids, antiepileptics, loop diuretics, thiazides, lithium, bisphosphonates or denosumab from the study (Figure 1a).

An elevated ACSC >10.5 mg/dL (hypercalcaemic) and ACSC between 8.9–10.5 mg/dL (normocalcaemic) with elevated parathormone (PTH) levels along with localisation of hyperfunctioning parathyroid gland by imaging modalities were considered diagnostic of PHPT. We analysed the data based on (a) symptoms – symptomatic PHPT – biochemical evidence of PHPT with symptoms of osseous, renal involvement or pancreatitis and asymptomatic PHPT – biochemical evidence of PHPT with lack of symptoms of PHPT (Figures 1a and 1b) ACSC – hypercalcaemic and normocalcaemic variant of PHPT. The latter subset was further subclassified into those with and without adequate 25OHD levels.

From the hospital, electronic records of all patients attending the endocrinology department outpatient, we retrieved subjects evaluated for serum calcium phosphorus, albumin, alkaline phosphatase, 25OHD and parathormone. We used the MDRD equation to calculate the estimated glomerular filtration rate (eGFR).[13] We excluded subjects with eGFR <60, those who were <18 and >80 years, if any one of the variables measured missing and duplicates from the analysis. We coded the subjects for analysis based on ACSC, PTH and 25OHD levels as four categories: (1) PHPT, (2) NCPHPT with adequate 25OHD level, (3) SHPT due to 25OHD deficiency (low or normal ACSC, raised PTH and low 25OHD) and (4) normal subjects with all three parameters being normal (Figure 1b). Patients from the study group were also included except for NCPHPT with low 25OHD levels for deriving the C/P ratio and PF index. We applied the new C/P ratio and PF index derived to our group of NCPHPT with low 25OHD levels to validate the indices derived. We used mmol (Standard International) whilst calculating CP and PF ratio.

Statistical analysis

To check the normality of data, one-sample Kolmogorov–Smirnov test was used. Continous variables Normal Mean ± SD is used to depict variables that are normally distributed. Median with interquartile range (IQR) is used for variables that do not conform to normality. Comparison of the biochemical variables between two groups is performed either by unpaired *t*-test or Mann–Whitney *U*-tests. Comparison amongst more than two groups was carried out by using the Kruskal–Wallis test followed by multiple comparison tests. Group difference means having $P < 0.05$ were considered statistically significant. Correlation amongst variables within groups is performed by Pearson's correlation coefficient, with $P < 0.05$ being considered significant. We performed statistical analysis using Statistical Package for Social Sciences (SPSS) version 25 (IBM Corp, Chicago, IL, USA).

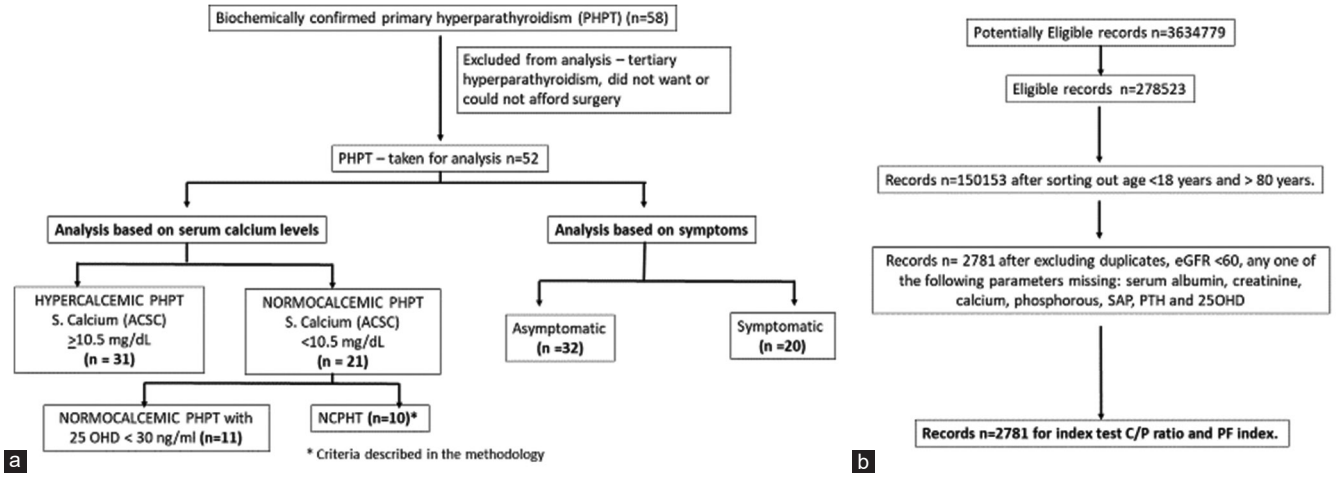


Figure 1: Flow chart for analysis of patients of PHPT (a). Flow chart of data mining and acquisition for evaluation of C/P ratio and PF index (b) ACSC = Albumin-corrected serum calcium; PHPT = Primary hyperparathyroidism; NCPHPT = Normocalcaemic primary hyperparathyroidism as per the criteria defined in the methodology; eGFR = Estimated GFR calculated using Modification of Diet in Renal Disease (MDRD) formula

Using MedCalc version 15·2 (Ostend Belgium), we carried out ROC curve analysis to test the diagnostic ability of C/P ratio and PF index. From the ROC plot, we derived the balance between sensitivity and specificity. Youden index was calculated to compare the diagnostic value of C/P ratio and PF index with PTH and serum calcium concentrations. A new ideal cut-off was obtained for both the C/P ratio and PF index. Further statistical analysis is performed to decide the superiority of the C/P ratio and PF index one over the other. $P < 0.05$ is taken as statistically significant. From the ROC analysis, we calculated the Youden index, positive predictive value and negative predictive value for all categories, namely PHPT, NCPHPT, SHPT and normal subjects.

RESULTS

The mean age at presentation was 54 ± 15 years; there were 37 (71%) females. There was no significant difference in the age between genders, symptomatic (20/52; 38.5%) versus asymptomatic (32/52; 61.5%) groups or between hypercalcaemic variant PHPT (31/52; 59%) versus normocalcaemic variant PHPT (21/52; 41%). In the group, none of them had an overt bone disease. Nephrocalcinosis was found in 3.8% (n = 2), ureteric stones in 5.8% (n = 3), renal stones in 28.8% (n = 15) and pancreatitis in 9.6% (n = 5) of patients. Statistically, there was no significant difference in various biochemical parameters between symptomatic and asymptomatic patients with PHPT except for the Cr/Cl ($P < 0·04$) and the 25OHD levels which were higher in the asymptomatic group ($P < 0·06$) (Table 1). In the whole group, there was a positive correlation between ACSC with SAP ($r = 0.312$; $P < 0.029$) and SAP and PTH ($r = 0.898$; $P < 0.0001$). Age negatively correlated with BMD, t-score at lumbar spine (LS) ($r = -0.482$; $P < 0.015$) and left and right hip ($r = -0.532$; $P < 0.007$).

Hypercalcaemic variant PHPT had much lower 25OHD levels, higher C/P ratio, PF index and weight of the adenomas compared to the normocalcaemic variant PHPT group (Table 1). The 25OHD levels were the highest in the NCPHPT group compared to the hypercalcaemic variant PHPT group ($P < 0·01$). A similar trend was followed with C/P ratio, LS-BMD and LS t-score. In the hypercalcaemic variant PHPT, ACSC correlated with creatinine ($r = 0.407$; $P < 0.026$) and weight of adenoma ($r = 0.742$; $P < 0.0001$), serum calcium with SAP ($r = 0.388$; $P < 0.038$) and SAP with PTH ($r = 0.939$; $P < 0.0001$). In the normocalcaemic variant PHPT, there was a negative correlation between PTH with serum calcium ($r = -0.535$; $P < 0.012$). Age correlated negatively with LS-BMD and t-score ($r = -0.733$; $P < 0.025$), left hip BMD and t-score ($r = -0.722$; $P < 0.28$) and right hip BMD and t-score ($r = -0.743$; $P < 0.02$). In patients with normocalcaemic variant PHPT with low 25OHD, serum calcium is negative with PTH ($r = -0.606$; $P < 0.048$).

In view of methodological differences in the estimation of various analytes in our previous work (1990)[6] and the present cohort (2021), in the NCPHPT group of the 2012 cohort the 25OHD levels were four times higher, with lower Ca/Cr ratio and lower SAP levels. The weight of the adenoma was lower by one-third compared to the 1990 cohort. In the hypercalcaemic PHPT group, the 25OHD level was three times higher with lower SAP and Ca/Cr ratio. The weight of the adenoma was one-fifth in the present cohort compared to that reported in our previous study (Table 2).

The mean ± SD and statistical significance of C/P ratio and PF index of various categories of PHPT, NCPHPT, SHPT and normal subjects are shown in box plots (Figure 2). Both indices help in differentiating various categories of patients studied. ROC curves were drawn to decide the cut-off values for identifying the C/P ratio and PF index in comparison with normal (Figures 2 and 3). Across all categories, PF index was more sensitive, specific and much superior compared to C/P ratio in the diagnosis of PHPT ($P = 0·02$), NCPHPT ($P = 0·03$) and SHPT ($P < 0·0001$). PF index (>25·8) was more sensitive (90%) and specific (96.51%) with an AUC of 0.96 and Youden index of 0.87 compared to C/P ratio (>0·211) (P 0·04) in differentiating NCPHPT from SHPT. We applied this PF index cut-off (>25·8) to our group of patients with NCPHPT with low Vitamin D and found the PF index (median with IQR) 36.92 (28.16–68.68; 40.52) validating the usefulness of PF index in differentiating NCPHPT from SHPT in these PHPT subjects with a proven histopathological diagnosis of parathyroid adenoma (Table 1). More details are available in *Online Supplementary Table 1, Online Supplementary Table 2 and Online Supplementary Figure 1*.

DISCUSSION

The clinical presentation of PHPT has undergone a paradigm shift. In the present cohort, the subjects were older, with none of them having an overt bone disease. The subjects have improved 25OHD levels, lower SAP and Ca/Cr ratio with smaller size and lower weight of the adenoma compared to 1990 cohort documented earlier. With improved vitamin D status and dietary calcium intake[8,13] we believe is thought to be the reason for

Table 1: The biochemical, bone mineral density variables of various categories of primary hyperparathyroidism patients

Variable	Hypercalcaemic PHPT (*n*=31)	Normocalcaemic PHPT (*n*=21)	Normocalcaemic PHPT with 25OHD <30 ng/mL (*n*=11)	Normocalcaemic PHPT with 25OHD >30 ng/mL (*n*=10)	Asymptomatic (*n*=32)	Symptomatic (*n*=20)
Serum Alb (g/dL)	3.80±0.73	3.77±0.84	3.71±1.05	3.83±0.57	3.86±0.66	3.66±0.93
Serum Cr (g/dL)	0.79 [0.66-0.88]	0.71 [0.63-0.97]	0.8 [0.63-1]	0.71 [0.61-0.88]	0.77 [0.64-0.88]	0.71 [0.63-0.9]
Serum CAL (g/dL)	12.18±1.98	9.25±1.17	9.07±1.32	9.45±1.01	11.01±2.01	11±2.58
ACSC (g/dl)	12.56±2.46	9.52±0.72	9.45±0.73	9.59±0.75	11.24±2.23	11.5±2.84
Serum phos (g/dL)	2.66±0.8	2.76±0.84	2.72±0.76	2.80±0.97	2.80±0.87	2.53±0.7
SAP (IU/L)	101 [80-146]	56.5 [70-123]	83 [63-127]	94 [78-119]	93 [77.75-116.25]	108 [72-161]
25OHD (ng/mL)	22.54±10.6	34.26±15.85	27.40±15.58	41.8±12.95b	29.79±14.05	23.25±13.5
PTH intact	202.7 [124.4-290]	140 [112-209]	140 [108-279]	140 [112.5-184.7]	168.55 [116.5-257]	194 [120-295]
Cr/Cl	91.40±25.13	86.75±25.34	87.7±32.28	85.72±16.35	85.55±23$	95.58±27.74
Ca/Cr ratio	0.07±0.11 (*n*=11)	0.04±0.05 (*n*=7)	0.04±0.05 (*n*=6)	0.02 (*n*=1)	0.08±0.13 (*n*=7)	0.04±0.07 (*n*=11)
C/P ratio	5.08±1.95	3.81±1.21	3.83±1.01	3.79±1.44	4.28±1.19	5.06±2.46
PF index	88.96 [46.88-145.72]	37.5 [27.69-74.47]	36.92 [28.16-68.7]	42.14 [26.9-79.56]	63.85 [36.04-92.67]	71.24 [34.4-171]
Weight (mg)	1280 [430-2800]	240 [106-640]	365 [80-620]	240 [107-810]	590 [324-1835]	595 [323-2800]
LS BMD	0.883±0.153 (*n*=16)	0.984±0.233 (*n*=9)	1.222±0.187 (*n*=3)	0.865±0.150 (*n*=6)	0.89±0.161 (*n*=18)	0.992±0.24
LS T-score	−2.46±1.4	−1.68±1.93	0.27±0.15	−2.65	−2.44	−1.5
Left hip BMD	0.794±0.109	0.829±0.202	0.915±0.284	0.915±0.284	0.800±0.156	0.826±0.13
Left hip T-score	−1.768±0.87	−1.4±1.6	−0.73	−0.73	−1.67	−1.54
Right hip BMD	0.801±0.120	0.823±0.197	0.910±0.250	0.910±0.250	0.800±0.162	0.833±0.12
Right hip T-score	−1.71±0.95	−1.64±1.57	−0.77	−0.77	−1.76	−1.49

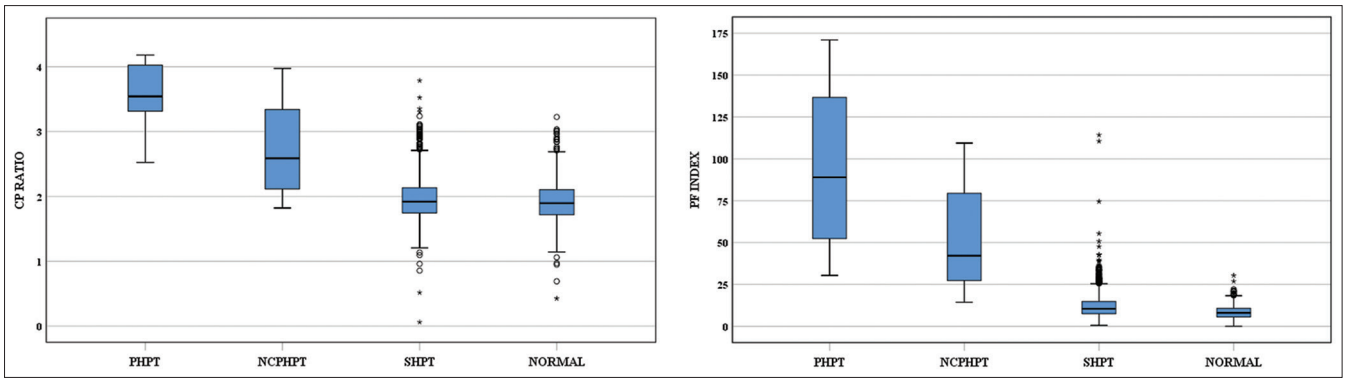
See text under "Results" for details regarding statistical significance on inter-group comparison
All values are mean±SEM. Comparison between two groups by Mann–Whitney U-test.
SEM=Standard error of the mean; PHPT=Primary hyperparathyroidism; Alb=Albumin; Cr=Creatinine; Cal=Calcium; ACSC=Albumin-corrected serum calcium, Phos=Phosphorous; SAP=Serum alkaline phosphatase; PTH MM=Parathyroid hormone Mid molecule; PTH-intact=Parathyroid hormone - intact molecule; Cr/Cl=Creatinine clearance; Ca/Cr=Calcium/creatinine ratio; 25OHD=25-hydroxyvitamin D

less severe manifest bone disease, lower SAP levels and Ca/Cr ratio and lowered size and weight of parathyroid adenoma. A systematic review of all published literature from developing countries shows that the symptomatic disease amongst PHPT patients varies between 79.6% and 95%.[14,15] Nevertheless, an asymptomatic presentation has been increasingly reported.[16] A retrospective review of medical records of PHPT patients in tertiary care centres from India showed that 18%–39% of patients are asymptomatic.[17,18] Subset analysis of data from the Indian PHPT registry showed that, although symptomatic disease predominates, the overall prevalence of asymptomatic patients has increased significantly from the first decade (2000–2009) to the second decade (2010–2019) (3% vs. 13%; $P = 0.003$).[19] Despite a rather small number in our study, nearly 62% (32/52) of our

Table 2: Comparison of biochemical parameters between 1990 cohort and 2021 cohort of patient with PHPT

Variable	Normocalcaemic PHPT		Hypercalcaemic PHPT	
	1995 (*n*=8)	2021 (*n*=21)	1995 (*n*=12)	2021 (*n*=31)
Age	37.25±5.3	52.86±3.48	36.83±4.25	55.06±2.68
Serum Alb (g/dL)	4.15±0.1	3.86±0.17	3.74±0.12	3.77±0.13
Serum Cr (mg/dL)	1.11±0.12	0.90±0.13	1.12±0.14	0.88±0.12
Serum Cal (mg/dL)	10.02±0.24	9.25±0.26	11.47±0.17	12.18±0.35
ACSC (mg/dL)	9.9±0.26	9.52±0.16	11.68±0.18	12.51±0.45
Serum phos (mg/dL)	3.00±0.24	2.69±0.18	3.20±0.29	2.52±0.16
SAP (IU/L)	352.63±90.85	101.01±12.3	473.42±70.11	150.79±37.72
25OHD (ng/mL)	7.94±1.94	34.19±3.46*	8.7±1.16	22.58±1.97
PTH-MM (pmol/l)	281.29±68.22		547.3±128	
Intact PTH (pmol/l)		194.30±40.93		343.11±89.17
Cr/Cl	49.8±4.43	86.47±6.12	35.83±6.79	91.37±4.75
Ca/Cr	1.37±0.22	0.03±0.02	1.71±0.18	0.07±0.03
Weight of gland (g)	3.75±1.6	0.22±0.08	9.96±3.55	1.73±0.41

See text under "Results" for details regarding statistical significance on inter-group comparison
All values are expressed as mean±SEM
SEM=Standard error of the mean; PHPT=Primary hyperparathyroidism; Alb=Albumin; Cr=Creatinine; Cal=Calcium; ACSC=Albumin-corrected serum calcium, Phos=Phosphorous; SAP=Serum alkaline phosphatase; PTH MM=Parathyroid hormone Mid molecule; PTH-intact=Parathyroid hormone - intact molecule; Cr/Cl=Creatinine clearance; Ca/Cr=Calcium/creatinine ratio; 25OHD=25-hydroxyvitamin D



	PHPT (*n*=31)	NCPHPT (*n*=10)	SHPT (*n*=1921)	Normal (*n*=771)
Age (years)*	55.09±14.8	59±15.17	49.35±14.16	54.03±14.07
ACSC*	3.13±0.61	2.39±0.18	2.31±0.11	2.34±0.11
PHOS (mmol)*	0.84±0.24	0.90±0.31	1.21±0.19	1.25±0.21
PTH (mmol)†	21.28 [13.06-30.45]	14.7 [11.81-19.4]	5.39 [3.93-7.46]	1.84 [3.01-5.63]
25OHD*	22.54±10.6	41.8±12.95	17.72±6.4	45.90±15.93
Calculated GFR*	91.37±25.57	85.11±17.22	105.26±18.07	99.16±17.40
C/P ratio*	4.03±1.77	3.03±1.38	1.96±0.33	1.92±0.32
PF index†	88.96 [46.88-145.72]	42.14 [26.9-76.5]	10.43 [7.55-14.8]	4.07 [5.59-10.73]

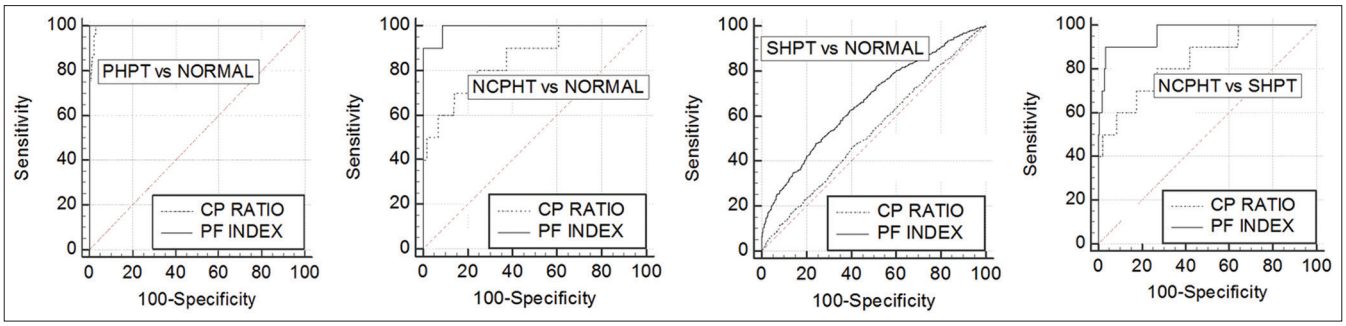
Figure 2: Box plot showing C/P ratio and PF index in various groups
*Data are presented as mean ± standard deviation
†Data are presented as median (interquartile range)
PHPT = Primary hyperparathyroidism; NCPHPT = Normocalcaemic primary hyperparathyroidism; SHPT = Secondary hyperparathyroidism; ACSC = Albumin-corrected serum calcium; Calcu GFR = Calculated glomerular filtration rate; C/P ratio = Calcium/phosphate ratio PF Index = Index is calculated by multiplying PTH (mM/L) by ACSC (mM/L) and dividing by serum phosphorous (mM/L) [(PTH (mM/L) × ACSC (mM/L)]/[serum phosphorous (mM/L)]

patients presented with asymptomatic disease, which is higher than what is now reported from India and reflects the changing pattern of presentation in India, just as seen in the Western world.

Data on the prevalence and presentation of the normocalcaemic variant have been sparse in the published literature. In an earlier study from India involving patients with PHPT, only 13.5% (7/52) of patients reported normocalcaemia.[20] Besides this, there are only a few cases reported about NCPHPT from India to date.[21-23] We have found that 41% (21/52) of our patients have normocalcaemia. Results from this study reiterate our earlier observation from a similar study done in 1990 of the very high prevalence of normocalcaemic variant[8] despite changing definitions with time.

Globally, in a study published as early as 1997, looking at population-based mammography screening on 5202 women from Sweden, only 28 of them (0.5%) were NCPHPT. This study was done before the current definition of NCPHPT came into vogue and 25OHD levels were not measured.[6] A community-based study involving 2364 subjects (all men), who were asymptomatic, showed a very low prevalence of NCPHPT (0.4%). They used a 25OHD value of 20 ng/mL as a cut-off to define sufficiency.[24] Swedish retrospective data involving 608 subjects aged between 25 and 64 years studied as part of the WHO MONICA Study in 1995 and later followed up in 2008 showed an increased prevalence of NCPHPT (11%) in 2008 compared to 1995 (3%). Serum 25OHD levels of more than 20 ng/mL were enough for inclusion in the study.[25] Interestingly, a Brazilian prospective study on 676 patients undergoing thyroid surgery looked at PTH and calcium levels and subclassified them variably based on 25OHD levels. The authors found the NCPHPT prevalence dropped from 4.4% to 0.74% when the 25OHD threshold changed from 20 ng/mL to 30 ng/mL, clearly highlighting the role of 25OHD.[26] The NCPHPT prevalence in our study population is very high compared to these studies. Our endocrine unit is a tertiary care referral centre with a special interest in metabolic bone diseases. Patient referral patterns may have influenced the prevalence.

Guo *et al*.[12] were the first to find the usefulness of the PF index in helping to differentiate NCPHPT from SHPT. Data from their study involved patients with 25OHD deficiency in all categories selected to derive at PF index. This we believe is an important confounding reason that would influence the value and makes its usefulness



Marker	PHPT versus normal		NCPHPT versus normal		SHPT versus normal		NCPHPT versus SHPT	
	C/P ratio	PF index	C/P ratio	PF index	C/P ratio	PF index	C/P ratio	PF index
Cut-off	>2.52	>30.29	>2.11	>14.33	>1.95	>10.76	>0.211	>25.8
Sensitivity (%)	100	100	80	100	46.64	47.84	80	90
Specificity (%)	96.89	100	75.88	91.05	59.01	75.5	73	96.51
AUC	0.99	1	0.85	0.99	0.53	0.66	0.94	0.96
Youden index	0.97	1	0.56	0.91	0.06	0.23	0.53	0.87
P	0.02		0.03		<0.0001		0.04	
PPV (%)	55.6	100	4.1	12.7	73.9	82.9	1.5	12
NPV (%)	100	100	99.7	100	30.7	36.9	99.9	100
Disease prevalence (%)	3.75	3.75	1.28	1.28	71.4	71.4	0.52	0.52
Accuracy (%)	97.01	100	75.93	91.16	50.18	55.75	73.04	96.48

Marker	Diagnosis of PHPT				Diagnosis of NCPHPT			
	Criterion	Sensitivity (%)	Specificity (%)	Youden index	Criterion	Sensitivity (%)	Specificity (%)	Youden index
PF index	>30.29	100	100	1	>14.33	100	91.05	0.91
Calcium	>2.62	100	98.96	0.99	>2.57	40	98.18	0.38
PTH	>9.18	100	99.87	1	>7.21	100	91.57	0.92
Calcium×PTH	>22.41	100	99.87	1	>22.41	100	97.15	0.97
Ca/P	>2.52	100	96.89	0.97	>2.11	80	75.88	0.56

Figure 3: ROC plots of C/P ratio and PF index of various categories
PPV=Positive predictive value; NPV=Negative predictive value; AUC=Area under curve; PF=Parathyroid function index; CP=Calcium-to-phosphate ratio; PHPT=Primary hyperparathyroidism; NCPHPT=Normocalcaemic PHPT, SHPT=Secondary hyperparathyroidism; PTH=Parathyroid hormone; Ca/P=calcium and serum phosphorous

questionable particularly when applied on patients to distinguish NCPHPT with low 25OHD level from SHPT. Furthermore, the method used by the authors to arrive at a single cut-off value of >34 as against four different values for different groups remains unclear. Whereas, we have carefully analysed our data by clearly selecting the groups that included those with (1) PHPT, (2) NCPHPT with adequate 25OHD levels and selected, (3) SHPT patients and (4) normal subjects as controls from our hospital registry. We calculated the PF index, compared various groups and arrived at cut-off values that could help differentiate these groups. We have then applied the newly derived cut-off value of PF index (>25.8) (SHPT vs. NCPHPT with adequate Vitamin D status) on our group of NCPHPT patients with low 25OHD levels and found it 100% correct in diagnosing PHPT (Table 1). Our data show that while the C/P ratio is largely unhelpful on its own as a measure to distinguish NCPHPT versus SHPT, the PF index was found useful, thereby helping us to differentiate between these two conditions without having to wait for a few weeks for correction of 25OHD status. This has enormous potential as a very practical and useful tool in clinical settings where compliance to outpatient remains a challenge. To the best of our knowledge, this is the first study that comprehensively evaluated PF index and Ca/P ratio by including patients with adequate 25OHD levels in PHPT and NCPHPT patients.

There was a lack of estimation of ionised calcium, Ca: Cr ratio and BMD in all subjects. The small sample size in our study could exaggerate the calculated proportions of all categories and subgroups. PHPT presentation has undergone a paradigm shift with a significant rise in asymptomatic and normocalcaemic presentation. While the C/P index categorically diagnoses PHPT, the PF index is more sensitive and specific, with a higher Youden index to differentiate NCPHPT from SHPT. Although correcting 25OHD levels and reassessing patients post-correction for PHPT remain a valid approach, the PF index could be an extremely useful tool to predict the same without the time lag and hence may hasten right patient care.

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Nil.

Conflicts of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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SUPPLEMENTARY MATERIAL

Supplementary Table 1: Biochemical, Bone mineral density variables of various categories of primary hyperparathyroidism patients analysed

	HYPERCALCEMIC PHPT (n =31)	NORMOCALCEMIC PHPT (n=21)	NORMOCALCEMIC PHPT WITH 25OHD < 30 ng/ml (n=11)	NCPHT WITH 25OHD > 30 ng/ml (n=10)	ASYMPTOMATIC n=32	SYMPTOMATIC n= 20
S.ALB *gm/dl*	3.80 ± 0.73	3.77 ± 0.84	3.71 ± 1.05	3.83 ± 0.57	3.86 ± 0.66	3.66 ± 0.93
S.CR *gm/dl*	*0.79 [0.66-0.88]*	*0.71 [0.63-0.97]*	*0.8 [0.63-1]*	*0.71 [0.61-0.88]*	*0.77 [0.64 - 0.88]*	*0.71 [0.63 - 0.9]*
S.CAL *gm/dl*	12.18 ± 1.98*[b]	9.25 ± 1.17	9.07 ± 1.32[a]	9.45 ± 1.01 [a]	11.01 ± 2.01	11 ± 2.58
ACSC *gm/dl*	12.56 ± 2.46*[b]	9.52 ± 0.72	9.45 ± 0.73 [a]	9.59 ± 0.75 [a]	11.24 ± 2.23	11.5 ± 2.84
S.PHOS *gm/dl*	2.66 ± 0.8	2.76 ± 0.84	2.72 ± 0.76	2.80 ± 0.97	2.80 ± 0.87	2.53 ± 0.7
SAP IU/L	*101 [80 - 146]*	*56.5 [70-123]*	*83 [63-127]*	*94 [78 - 119]*	*93 [77.75 - 116.25]*	*108 [72-161]*
25 OHD *ng/ml*	22.54 ± 10.6*[a]	34.26 ± 15.85	27.40 ± 15.58 [a]	41.8 ± 12.95[b]	29.79 ± 14.05	23.25 ± 13.5
PTH intact	*202.7 [124.4-290]**	*140 [112 - 209]*	*140 [108-279]*	*140 [112.5-184.7]*	*168.55 [116.5 - 257]*	*194 [120-295]*
Cr/Cl	91.40 ± 25.13	86.75 ± 25.34	87.7 ± 32.28	85.72 ± 16.35	85.55 ± 23[$]	95.58 ± 27.74
Ca: Cr RATIO	0.07 ± 0.11 {11}	0.04 ± 0.05{7}	0.04 ± 0.05 {6}	0.02{1}	0.08 ± 0.13 {7}	0.04 ± 0.07 {11}
C/P RATIO	5.08 ± 1.95*[b]	3.81 ± 1.21	3.83 ± 1.01 [a]	3.79 ± 1.44 [a]	4.28 + 1.19	5.06 ± 2.46
PF INDEX	*88.96 [46.88-145.72] *[b]*	*37.5 [27.69 - 74.47]*	*36.92 [28.16-68.7] [a]*	*42.14 [26.9-79.56] [a]*	*63.85 [36.04 - 92.67]*	*71.24 [34.4 - 171]*
WEIGHT *mgs*	*1280 [430 -2800] *[b]*	*240 [106 - 640]*	*365 [80-620] [b]*	*240 [107-810] [a]*	*590 [324-1835]*	*595 [323 - 2800]*
LS BMD	0.883 ± 0.153 {16}	0.984 ± 0.233 {9}	1.222 ± 0.187 {3}	0.865 ± 0.150 {6}	0.89 ± 0.161 {n=18}	0.992 ± 0.24
LS T SCORE	-2.46 ± 1.4	-1.68 ± 1.93	0.27 ± 0.15	-2.65	-2.44	-1.5
L HIP BMD	0.794 ± 0.109	0.829 ± 0.202	0.915 ± 0.284	0.915 ± 0.284	0.800 ± 0.156	0.826 + 0.13
L HIP T SCORE	-1.768 ± 0.87	-1.4 ± 1.6	-0.73	-0.73	-1.67	-1.54
R HIP BMD	0.801 ± 0.120	0.823 ± 0.197	0.910 ± 0.250	0.910 ± 0.250	0.800 ± 0.162	0.833 +0.12
R HIP T SCORE	-1.71 ± 0.95	-1.64 ± 1.57	-0.77	-0.77	-1.76	-1.49

PHPT=Primary Hyperparathyroidism; Serum(S)ALB=Serum Albumin; S.CRE=S. Creatinine; S.CAL=S. Calcium; ACSC=Albumin Corrected Serum Calcium; S.PHOS=S. Phosphorous; SAP=Serum Alkaline Phosphatase; PTH=Parathyroid Hormone; Cr/Cl=Creatinine Clearance; Ca/Cl=Calcium/Creatinine Ratio; CP ratio=Calcium Phosphate Ratio; PF-index=Parathyroid Function Index; LS BMD=Lumbar Spine Bone Mineral Density; L HIP BMD=Left Hip BMD; R HIP BMD=Right Hip BMD.

All values are mean + SD. Values in italics are median and IQR within square brackets. Values in flower brackets denote the number tested. Values in the box with thin lines – comparison between Hypercalcemic PHPT and Normocalcemic PHPT was by Mann- Whitney U test.

*denotes the significant difference in the row between the groups. Shaded box – data of Normocalcemic PHPT – sub-classified as normocalcemic PHPT with 25OHD <30 ng/ml and NCPHPT (25OHD >30 ng/ml as defined by recent definition).9 Values between these groups were compared with hypercalcemic PHPT by the Kruskal Wallis test. Values in each row having similar alphabetic superscripts do not differ significantly. Values in Bold box – symptomatic PHPT and asymptomatic PHPT comparison by Mann- Whitney U test. [$]denotes a significant difference in the row between the groups.

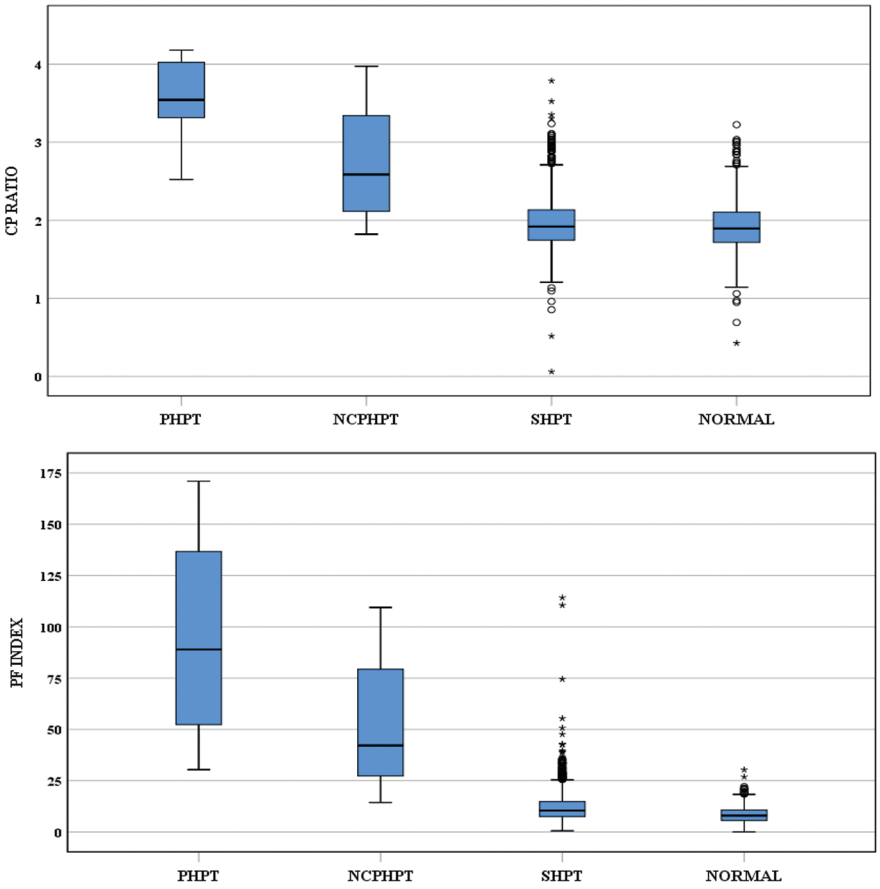
SUPPLEMENTARY MATERIAL

Supplementary Table 2: Comparison of biochemical parameters between 1990 cohort and 2021 cohort of patients with PHPT

	NORMICALCEMIC PHPT		HYPERCALCEMIC PHPT	
	1995 (n=8)	**2021 (n=21)**	**1995 (n=12)**	**2021 (n=31)**
AGE	37.25 ± 5.3	52.86±3.48 [d]	36.83± 4.25	55.06± 2.68 [b]
S.ALB *gm/dl*	4.15 ± 0.1	3.86± 0.17	3.74± 0.12	3.77± 0.13
S Cr *mg/dl*	1.11 ± 0.12	0.90 ± 0.13 [c]	1.12± 0.14	0.88± 0.12[d]
S.Cal *mg/dl*	10.02 ± 0.24	9.25± 0.26	11.47± 0.17	12.18± 0.35
ACSC mg/dl	9.9 ± 0.26	9.52± 0.16	11.68± 0.18	12.51± 0.45
S Phos *mg/dl*	3.00 ± 0.24	2.69± 0.18	3.20± 0.29	2.52 ± 0.16[$]
SAP *IU/L*	352.63 ± 90.85	101.01± 12.3 [b]	473.42± 70.11	150.79± 37.72*
25 OHD3 *ng/ml*	7.94 ± 1.94	34.19± 3.46*	8.7± 1.16	22.58± 1.97*
PTH-MM *pmol/l*	281.29 ± 68.22		547.3± 128	
Intact PTH *pmol/l*		194.30± 40.93		343.11± 89.17
Cr/cl	49.8 ± 4.43	86.47± 6.12 [a]	35.83± 6.79	91.37± 4.75*
Ca/Cr	1.37 ± 0.22	0.03± 0.02*	1.71± 0.18	0.07± 0.03*
Wt of gland *gms*	3.75 ± 1.6	0.22± 0.08 [e]	9.96± 3.55	1.73± 0.41*

PHPT=Primary Hyperparathyroidism; Serum(S)ALB=S. Albumin; S.CRE=S. Creatinine; S.CAL= S. Calcium; ACSC=Albumin Corrected Serum Calcium; S.PHOS=Phosphorous; SAP=Serum alkaline phosphatase; PTH MM=Parathyroid hormone Mid molecule; PTH-intact=parathyroid hormone – intact molecule; Cr/Cl=Creatinine clearance; Ca/Cl=Calcium/creatinine ratio; CP ratio=Calcium phosphate ratio; PF-index=Parathyroid function index, All values are mean + SEM. Comparison between two groups by Mann- Whitney U test.
*P <0.0001, [a]P <0·001, [b]P<0·002, [c]P <0·02, [d]P <0·03, [e]P < 0.004, [$]$P$ <0.05 denotes significant difference in the row between the groups

SUPPLEMENTARY MATERIAL



	PHPT (n=31)	NCPHPT (n=10)	SHPT (n=1921)	NORMAL (n=771)
AGE	55·09 ± 14·8	59 ± 15·17	49·35 ± 14·16	54·03 ± 14·07
ACSC	3·13 ± 0·61	2·39 ± 0·18	2·31 ± 0·11	2·34 ± 0·11
PHOS mmol	0·84 ± 0·24	0·90 ± 0·31	1·21 ± 0·19	1·25 ± 0·21
PTH mmol	*21·28 [13·06-30·45]*	*14·7 [11·81-19·4]*	*5·39 [3·93-7·46]*	*1·84 [3·01-5·63]*
25OHD	22·54 ± 10·6	41·8 ±12·95	17·72 ± 6·4	45·90 ± 15·93
Calcu GFR	91·37 ± 25·57	85·11 ± 17·22	105·26 ± 18·07	99·16 ± 17·40
CP RATIO	4·03 ± 1·77	3·03 ± 1·38	1·96 ± 0·33	1·92 ± 0·32
PF INDEX	*88·96 [46·88-145·72]*	*42·14 [26·9-76·5]*	*10·43 [7·55-14·8]*	*4·07 [5·59-10·73]*

Supplementary Figure 1: Box plot showing CP ratio and PF index in various groups.
PHPT=Primary hyperparathyroidism; NCPHPT=Normocalcemic primary hyperparathyroidism; SHPT=Secondary hyperparathyroidism; ACSC= Albumin Corrected Serum Calcium, Calcu GFR=Calculated glomerular filtration rate. CP ratio=Calcium phosphate ratio (Calcium/Phosphate ratio), PF- index is calculated by multiplying PTH (mmol/L) by ACSC (mmol/L) and divided by serum phosphorous (mmol/L) [PTH*ACSC/S. Phosphorous]. All values are mean + SD. Values in italics denote median and IQR in square brackets. The values in each row are significantly different from each other P<0·0001 by the Kruskal Wallis test.