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# A pilot study on hypothalamo-pituitary-adrenocortical axis in primary hyperparathyroidism

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***Background & objectives***: **Parathormone (PTH) and calcium, both have been shown to stimulate adrenal steroidogenesis in animal models and *in vitro* experiments. This is attributed to structural similarity between 15-25 amino acid region of the parathyroid hormone (PTH) and 1-11 amino acid region of adrenocorticotropin (ACTH). However, there are no *in vivo* human data regarding the effect of PTH-calcium axis on adrenocortical function.**

***Materials*: Ten patients with primary hyperparathyroidism underwent evaluation for cortisol dynamics including 0800 h and 2000 h plasma cortisol on day 1, cortisol response to insulin induced hypoglycaemia (IIH) on day 2, and 1 mg overnight dexamethasone suppression test (ONDST) on day 4. Serum aldosterone was also measured at 0800 h in fasting state on salt *ad libitum* for three days. These parameters were repeated 3 months after curative parathyroidectomy.**

***Results*: Basal plasma cortisol level at 0800 h and 2000 h were within upper normal range and loss of circadian rhythm in cortisol secretion was observed in half and forty per cent of patients had non-suppressibility with ONDST. The defined peak cortisol response to insulin induced hypoglycaemia (>550 nmol/l) was achieved in all and nearly one third of patients had exaggerated response (>2000 nmol/l). After curative parathyroidectomy, the abnormalities in circadian rhythm and non-suppressibility with ONDST continued to prevail in 40 per cent of patients. The peak cortisol response to IIH showed a decrement but remained higher than normal. No correlation was observed between circulating parathyroid hormone and calcium with cortisol levels. Serum aldosterone was in upper normal range pre - and postoperatively, though it decreased postoperatively, but it could not attain a statistical significance ($P = 0.5$).**

***Interpretation & conclusion*: Abnormalities in hypothalamo-pituitary-adrenocortical axis in primary hyperparathyroidism do occur, however these are inconsistent and do not recover in majority of patients even after 3 months of curative parathyroidectomy.**

**Key words** Cortisol - hyperparathyroidism - osteoporosis

Parathyroid hormone (PTH), one of the main calcium regulating hormone acts primarily on bone and kidney through the cyclic adenosine mono phosphate (cAMP) pathway. Of the sequence of 84 amino acid residues in human PTH (PTH), the 1-34 amino terminal peptide is considered to be the minimal length required for its maximal activity[1]. The active PTH peptide contains a region primarily responsible

for binding to the receptors and a small but distinct region responsible for its hormone action. In addition to its effect on the main target organs like kidney and bone, PTH also stimulate adrenocortical steroid production *in vivo* and *vitro* in various animal models[1-2]. This effect of PTH is attributed to structural similarity between 15-25 amino acid of PTH and 1-11amino acid of adrenocorticotrophin (ACTH) and/or by an indirect action through hypercalcaemia[2]. Both high concentrations of PTH (1-34) and calcium stimulate adrenal cAMP enhance cortisol and aldosterone secretion *in vitro* as well as *in vivo* in animal models and in human dispersed adrenocortical cells[2-8].

The relationship between PTH and renin-angiotensin-aldosterone system has been a matter of debate in patients with primary hyperparathyroidism and still remains unresolved[7-8].

Primary hyperparathyroidism is characterized by inappropriately elevated iPTH in presence of hypercalcaemia and both these may alter the functional status of hypothalmo-pituitary-adrenocortical (HPA) axis in these patients. Therefore, this study was planned to investigate the HPA axis in patients with primary hyperparathyroidism before and after curative surgery.

## Material & Methods

Fifteen consecutive patients with PHPT attending Endrocrinology OPD at Postgraduate Institute of Medical Education & Research, Chandigarh were recruited during the period of January 2005 to December 2006 and finally 10 patients were evaluable at the end of the study. The study was approved by Institute's ethics committee. A written informed consent was obtained from all the patients. Patients with high serum calcium (> 11mg/dl), inappropriately elevated PTH in the presence of normal renal functions and demonstration of a parathyroid adenoma on histopathology were included in the study.

Patients with concurrent medical illness, renal failure, pregnancy, fracture of less than 2 wk duration (to endure with stress) and on antipsychotic drugs, oral contraceptives or glucocorticoids were excluded.

Calcium profile [Calcium (Ca), inorganic phosphate ($PO_4$), alkaline phosphatase and albumin] in fasting state and without tourniquet application was measured on three consecutive days and corrected serum calcium was derived after adjusting for serum albumin. Also, before testing it was ensured that the patient should be taking a normal dietary sodium for three days, are well hydrated and should not have serum albumin of <2.5 gm/dl, the variables known to affect the plasma cortisol and aldosterone levels. On day 1 at 2000 h plasma cortisol sample was taken. On day 2 at 0800 h after an overnight fast, samples were taken for iPTH, cortisol and aldosterone and patients were subjected to insulin induced hypoglycaemia (IIH). Blood samples for cortisol were obtained at -20, 0, 30, 60, 90 and 120 min after administration of regular insulin (human Actrapid, Novo-Nordisk, Denmark) at a dose of 0.1-0.2 U/kg IV bolus. A glucose level of ≤ 40mg/dl was considered as an appropriate stimulus for sufficient ACTH-cortisol release. On day 3 at 2300 h patients received 1mg oral dexamethasone and next day (day 4) at 0800 h blood sample for cortisol was drawn. The reference range for cortisol at 0800 h, 2000 h, were 400-600 nmol/l, and 200-400 nmol/l respectively. Loss of circadian rhythm was defined by failure of 2000 h cortisol to decrease by <50 per cent of morning cortisol. Peak cortisol of >550 nmol/l was defined as optimal response to IIH. While a non-suppressible overnight dexamethasone test was defined as 0800 h cortisol value of >140 nmol/l.

Cure was defined as normalization of serum calcium, decrease in iPTH by fifty percent from the baseline and histopathologically verified parathyroid adenoma. Serum calcium, iPTH and cortisol dynamics parameters were repeated after 3 months of curative surgery.

*Assay methods*: Intact parathyroid hormone (iPTH) was measured by solid phase two site chemiluminescent enzyme-labelled immunometric assay. The reference range for iPTH in this study was 10-69 pg/ml with the intra- and inter-assay coefficient of variation being 6.3 and 8.6 per cent respectively[9]. Plasma cortisol was measured using an in house radioimmunoassay[10]. The analytical sensitivity of the assay was 5 nmol/l with the intra- and inter-assay coefficient of variation 8.5 and 5.7 per cent, respectively[10]. Aldosterone was assayed by Coat-A-Count solid phase radioimmunoassay. Coat-A-Count is a solid-phase radioimmunoassay, based on aldosterone specific antibody immobilized to the wall of a polypropylene tube. The reference range for assay was 4-31 ng/dl. The analytical sensitivity of the assay was 11 pg/ml (pg/ml÷ 10= ng/dl) with the intra- and inter-assay coefficient of variation 2.3 and 3.8 per cent respectively[11].

*Statistical analysis*: Entire data was subjected to suitable statistical analysis. The patients acted as their own controls and pre-treatment and 3 months post-surgery parameters were compared using the Wilcoxan rank

test. Correlation of these parameters to serum iPTH and serum calcium will be studied using Spearman's rank correlation. All tests of statistical significance were two tailed and a $P \leq 0.05$ was considered significant. Results are expressed in median with inter-quartile range.

## Results

Out of 15 patients screened, five patients were not included in final analysis as 4 were lost to follow up and one had multiple endocrine neoplasia-1 (MEN-1). These ten patients (7 women) had a mean (± SD) age of 32.8±13.1 yr. The mean (±SD) lag time from first reported symptom to the time of evaluation of PHPT was 2.46±1.79 yr with a range from 1 month to 6 yr. The clinical signs and symptoms of primary hyperparathyroidism are summarized in Table I. All patients underwent curative parathyroidectomy. Details of pre - and post operative calcium, phosphate, alkaline phosphatase, iPTH and urinary calcium are given in Table II. Serum 25 (OH) vitamin D levels at baseline was 15.8±11.0 ng/ml with a range of 5 to 38.4 ng/ml (normal range 9-37.6 ng/ml). Out of these ten patients, four patients were vitamin D deficient.

The preoperative basal 0800 h and 2000 h serum cortisol expressed as median with interquartile range (IQR) of 25$^{th}$-75$^{th}$ percentile were 509.5 (384.3-533.8) nmol/l and 226.5 (155.5-581.3) nmol/l respectively with loss of circadian rhythm in half of the patients (Table III). Serum cortisol levels were non-suppressible in forty per cent of patients after 1mg overnight dexamethasone. The defined peak cortisol response to IIH (>550 nmol/l) was observed in all and 40 per cent of patients had exaggerated response (>2000 nmol/l). Three months postoperatively basal 0800 h and 2000 h serum cortisol were 453.5 (362.8-619.5) nmol/l and 255.5 (139.0-828.0) nmol/l with non-restoration of circadian variation in half of the patients. Moreover serum cortisol remained non-suppressible after 1mg ONDST in forty per cent of patients postoperatively. Peak cortisol response to IIH was preserved in all, and only 2 (20%) patients had exaggerated response (>2000 nmol/l). The peak cortisol response to IIH failed to show any correlation with serum calcium and iPTH in both pre - and post operative period ($P$= 0.59, r=0.19, $P$=0.38, r= 0.31, $P$=0.27, r=0.39, $P$=0.63, r=0.17) respectively.

**Table I.** Clinical profile of patients with primary hyperparathyroidism pre- and post surgery (n=10)

| Clinical profile | Pre operative | Post operative |
|---|---|---|
| Bone pains | 6 | Nil |
| Bone swelling/deformity | 4 | Nil |
| Fracture | 5 | Nil |
| Renal colic | 3 | 1 |
| Graveluria | 1 | 1 |
| Anorexia | 2 | Nil |
| Weight loss | 5 | Nil |
| Nausea/vomiting | 2 | Nil |
| Constipation | 3 | Nil |
| Loss of teeth | 4 | Nil |
| Pruritis | 1 | Nil |
| Weakness/fatiguability | 7 | Nil |
| Acid peptic disease | 2 | Nil |
| Pancreatitis | 2 | Nil |
| Neuropsychiatric manifestations | 3 | Nil |
| Hypertension | 2 | 2 |
| Systolic BP (mg Hg, mean ± SD) | 130.0 ± 18.8 | 128.8 ± 17.9 |
| Diastolic BP | 83.9 ± 10.48 | 82.2 ± 9.16 |

**Table II.** Biochemical profile of patients with primary hyperparathyroidism pre- and post surgery (n=10)

| Parameters | Preoperative | Postoperative |
|---|---|---|
| Serum calcium (mg/dl) [8.5-11.4] | 10.1 ± 0.9 | 9.0 ± 0.7* |
| Serum phosphate (mg/dl) [3-5] | 2.9 ± 0.9 | 3.5 ± 0.4** |
| Serum alkaline phosphatase (KAU) [3-13] | 57.4 ± 48.8 | 18.6 ± 8.6* |
| Serum iPTH (pg/ml) (10-69) | 1141.8 ± 717.4 | 96.8 ± 44.5** |
| Urine calcium (mg/24 h) [100-400 mg] | 369.0 ± 196.2 | 136.0 ± 76.8* |
| Urine phosphate (mg/24 h) [1000-2000 mg/24 h] | 825.0 ± 290.7 | 439.8 ± 231.3** |
| Urine creatinine (mg/24 h) | 802.6 ± 302.5 | 827.0 ± 23.3 |

Values are mean ± SD (n=10); Values shown in [ ] are reference range
*P**<0.05, ****<0.01 compared to preoperative value

**Table III.** Cortisol profile in patients with primary hyperparathyroidism (n=10)

| Parameters | Preoperative cortisol (nmol/l) Median (IQR 25-75$^{th}$ percentile) | Postoperative cortisol (nmol/l) Median (IQR 25-75$^{th}$ percentile) | *P* value |
|---|---|---|---|
| 0800 h | 509.5 (384.3-533.8) | 453.5 (362.8-619.5) | 0.87 |
| 2000 h | 226.5 (155.5-581.3) | 255.5 (139.0-828.0) | 0.28 |
| Loss of circadian rhythm | 50 per cent of patients | 50 per cent of patients | |
| Insulin induced hypoglycaemia (IIH) | | | |
| 0 min | 532.5 (368.3-642.5) | 500.0 (392.5-592.5) | 0.72 |
| Peak cortisol | 915.0 (800.3-4120.0) | 970.0 (780-1174.0) | 0.38 |
| Overnight dexamethasone suppression (dexamethasone 1 mg at 2300 h) | | | |
| 0800 h | Non-suppressible 40 per cent of patients | Non-suppressible 40 per cent of patients | 0.87 |

The mean (±SD) serum aldosterone was 25.06± 35.20 ng/dl preoperatively (normal range 4-31 ng/dl). Three month after the surgery aldosterone levels decreased to 18.03± 8.31 ng/dl ($P$ <0.5).

## Discussion

This study shows that patients with primary hyperparathyroidism have alterations in HPA axis in form of loss of circadian rhythm of cortisol secretion, exaggerated cortisol response to insulin induced hypoglycaemia and failure to suppress cortisol after dexamethasone. Moreover, these alterations in HPA axis did not recover even after 3 months of curative parathyroidectomy.

Primary hyperparathyroidism is characterized by both increased levels of PTH and calcium and both of these have been shown to stimulate adrenocortical steroid production in various *in vitro* and *in vivo* studies[2-8]. However, there are no human studies available on alterations in HPA axis in patients with primary hyperparathyroidism. The levels of ACTH and cortisol peaks in the early morning and falls during the day to reach a nadir at about midnight[12]. On the contrary PTH level starts increasing around 2200 h and reaches its peak by midnight[1]. The loss of circadian rhythm in cortisol secretion was a consistent observation in half of the patients in our study and this may be attributed to increased levels of PTH and calcium or denovo stress due to chronic disease resulted in persistent activation of HPA axis.

Suppressibility of cortisol after dexamethasone is a sensitive test to assess the integrity of HPA axis[13-14] and more than one third of the patients had non-suppresible cortisol in this study. The non-suppressibility of 0800 h cortisol after dexamethasone is not only observed in endogenous Cushing syndrome but also in various non-endocrine disorders including obesity, alcoholism and depression. Primary hyperparathyroidism is not only a chronic stressful state but these patients also have subtle mood abnormalities. However, in our study, patients with evident depressive disorders were excluded and none was obese. Therefore, nonsuppressibility of cortisol after dexamethasone in these patients is a result of dysregulated HPA axis.

Hypoglycaemia is a potent stimulus for release of catecholamines, cortisol and growth hormone[15]. However studies in healthy adults have shown increase in PTH in response to IIH, whereas no change in PTH has been reported in patients with PHPT[16-18]. This may be due to already maximal secretion of PTH by the autonomous parathyroid gland[16]. However, there are no data regarding the concurrent increase or decrease in secretion of cortisol to IIH in these patients. In our study we observed nearly fourfold increase in plasma cortisol in response to IIH preoperatively and it still remained higher postoperatively. This could be explained by persistent hypercalcaemia and increase in ACTH in response to hypoglycaemia. This could be explained by activation of HPA axis by hypercalcaemia and /or PTH and it takes time to recover.

The abnormalities of cortisol dynamic persisted even after 3 months of successful surgery, suggests that elevated PTH and serum calcium levels alone do not explain all the observed abnormalities. Therefore some unidentified putative factors may be responsible or once HPA axis is dysregulated, it may take a longer time to recover.

It is known that one third of the patients with PHPT are hypertensive though the mechanism remains unclear. A study by Berrini *et al*[8] described renin angiotensin - aldosterone axis in patients with PHPT before and after surgery and demonstrated that PTH weakly correlated with plasma renin activity and had no correlation with serum aldosterone. In our study we did not observe any correlation between aldosterone and PTH-calcium axis.

Our study may translate into some clinical implications if substantiated in large number of patients namely osteoporosis and hypertension, which is usually an accompaniment in patients with primary hyperparathyroidism. Symptomatic PHPT is usually associated with decreased bone mineral density (BMD) which is more marked in cortical bone as compared to trabecular (spine) bone. However, with advanced disease, trabecular bone is also severely affected, as it is metabolically more active than cortical bone. Hypercortisolemia is also an important predisposing factor for osteoporosis[19] and if HPA axis is activated in patients with PHPT, it may contribute to further decrease in bone mineral density. Similarly, hypertension is also frequent in patients with PHPT and may be partly contributed by hypercortisolemia.

The study has following limitations including lack of control group comprising of non-parathyroid neck surgery to substantiate or refute altered cortisol dynamics were due to increased levels of PTH and calcium or merely a surgical stress. Serial measurement

of ACTH and PTH and IL-6 , a marker of stress , would have been of help to define the genesis of alteration in HPA axis.

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