COMPARISON OF METABOLIC AND CHARACTERISTIC FEATURES OF PRIMAY HYPERPARATHYROIDISM PATIENTS WITH DIFFERENT INTACT PTH LEVELS

By

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ABSTRACT OF THE THESIS

COMPARISON OF METABOLIC AND CHARACTERISTIC FEATURES OF PHPT PATIENTS WITH DIFFERENT INTACT PTH LEVELS

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This study is to compare primary hyperparathyroidism (PHPT) patients with different iPTH levels for serum calcium and other laboratory values. In this retrospective study, 212 patients who presented at Robert Wood Johnson Hospital were assessed for metabolic and characteristic features of PHPT. Patients were divided into two groups according to their serum intact parathyroid hormone (iPTH) levels. Student t- tests were used to compare the two groups for differences in age, body mass index (BMI), adenoma weight, and laboratory test values which included serum calcium, iPTH, 25OHD, lipid panel, serum creatinine, ALP, and 24 hr. urinary calcium. Pearson`s correlation coefficient was used to assess the relationship. Of the 212 PHPT patients, 100 (17 males and 83 females) were classified as m-iPTH group (iPTH below 140 pg/ml), whereas 112 patients (25 males and 87 females) were classified as h-iPTH group (iPTH above 140 pg/ml). Higher-iPTH patients are younger than m-iPTH patients. No statistic significant differences in BMI, T-cholesterol and TG were found between the m-iPTH and h-iPTH groups. Higher-

iPTH patients compared with m- iPTH, had slightly but significantly higher calcium, lower 25OHD, lower HDL, higher ALP, and very close to have higher adenoma weight. Additionally, we found iPTH was positive correlated with serum calcium, adenoma weight, and triglyceride (TG) levels, and negatively correlated with HDL and 25OHD. Intact PTH did not correlate with BMI and T- cholesterol levels. Furthermore, 24 hr. urinary calcium and serum ALP were positively associated with iPTH levels but not significantly ($P = 0.08$, $P = 0.09$) respectively. These correlations were independent of serum calcium and 25OHD levels except TG was dependent of reduced 25OHD, while serum ALP was dependent of calcium levels. These findings from our analysis consistent with previous studies suggesting iPTH levels correlated metabolic syndrome. Additionally, our results suggest that h- iPTH patients tend to be younger ones, with lower HDL, lower 25OHD, and higher ALP. While the underline mechanisms for these changes are unclear, we speculate that the elevated iPTH levels might decrease HDL directly or indirectly through increasing insulin resistance by weight gain, and increases ALP through PTH receptors on osteoblasts. This study supported our hypothesis that iPTH levels are an important factor to contribute in the management of PHPT patients. Intact PTH levels, lipid panel, 25OHD, ALP in addition to calcium levels might also need to be considered in the therapeutic decision for PHPT patients.

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Chapter 1

Introduction

Endocrinology is the branch of physiology and medicine concerned with glands and hormones. The endocrine system consists of glands, hormones, hormone-receptors, and hormone producing tissues. These glands comprise pituitary, adrenal, thyroid, pineal, thymus, and parathyroid glands. All of these glands are separated from each other but are working together within the whole body to cover several functions such as metabolism, reproduction, movement, and sensory perception (Fig.1) [1]. Endocrine hormones circulate through the blood stream to reach their target tissues. Some of them are classified as local hormones that work on the nearby cells (paracrine) or on the same cells which are secreting them (autocrine) and thus do not enter the bloodstream (Fig. 2) [2]. Hormones are classified into groups depending on their molecular structure. These groups include polypeptides, steroids, and amines, where some are water- soluble hormone and the others are lipo-soluble hormones [1].

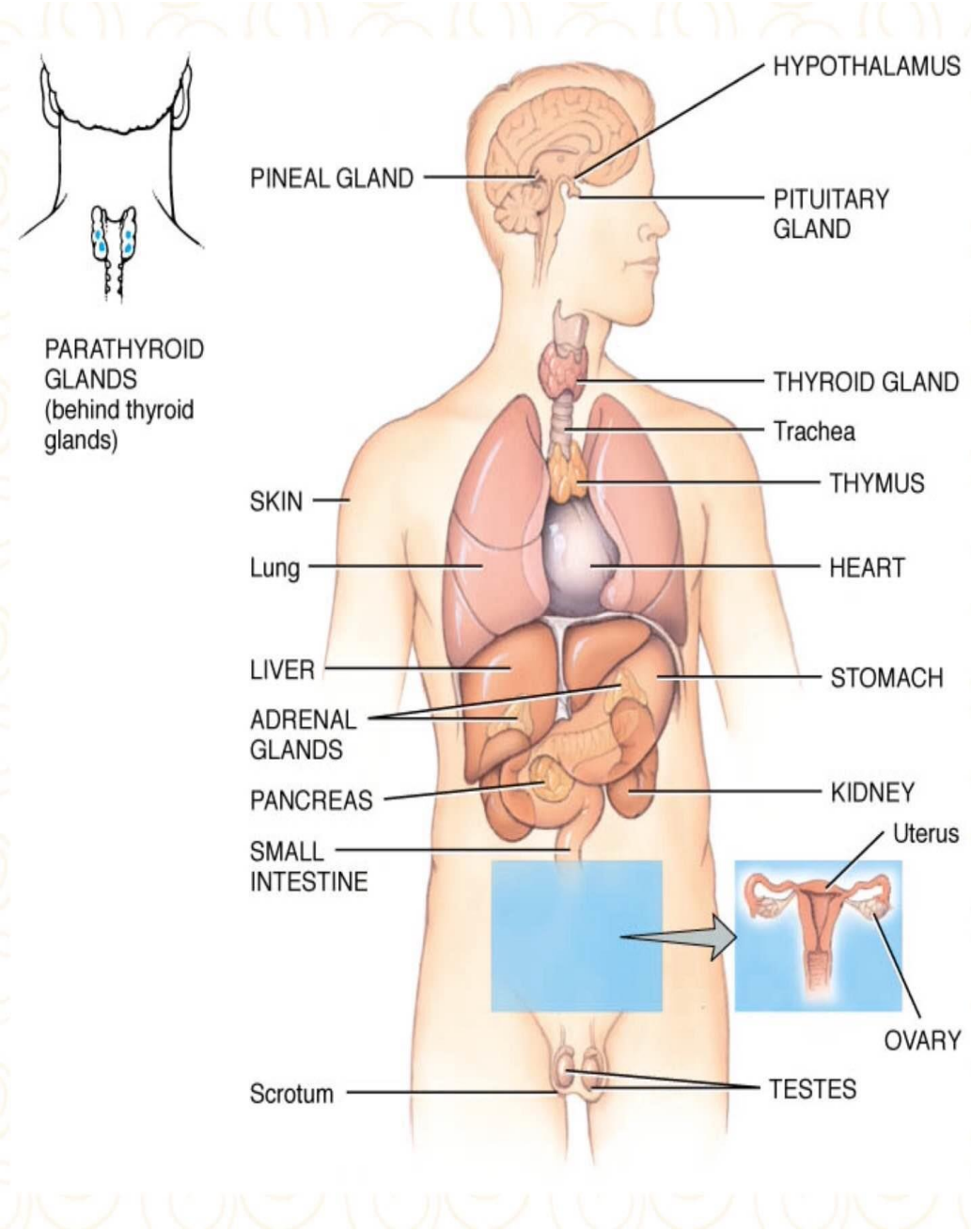


Figure 1: Anatomical diagram of the major endocrine glands, taken from [3]

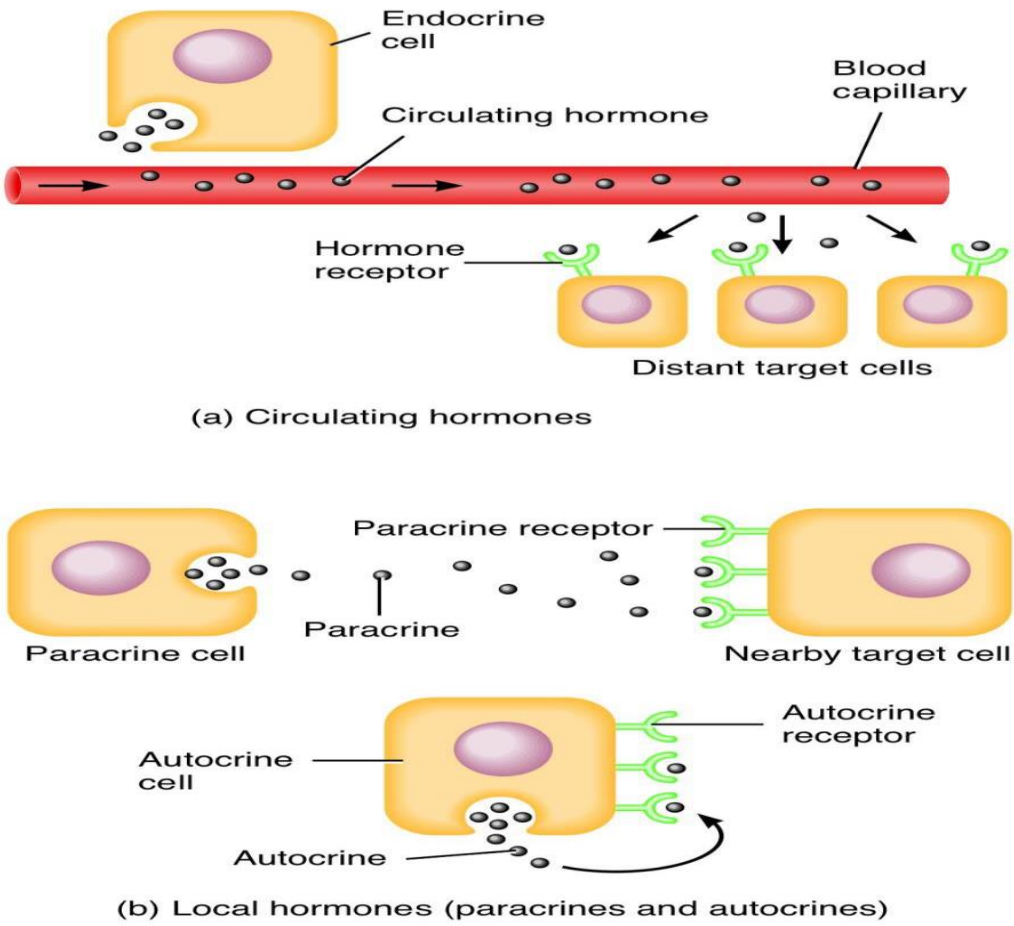


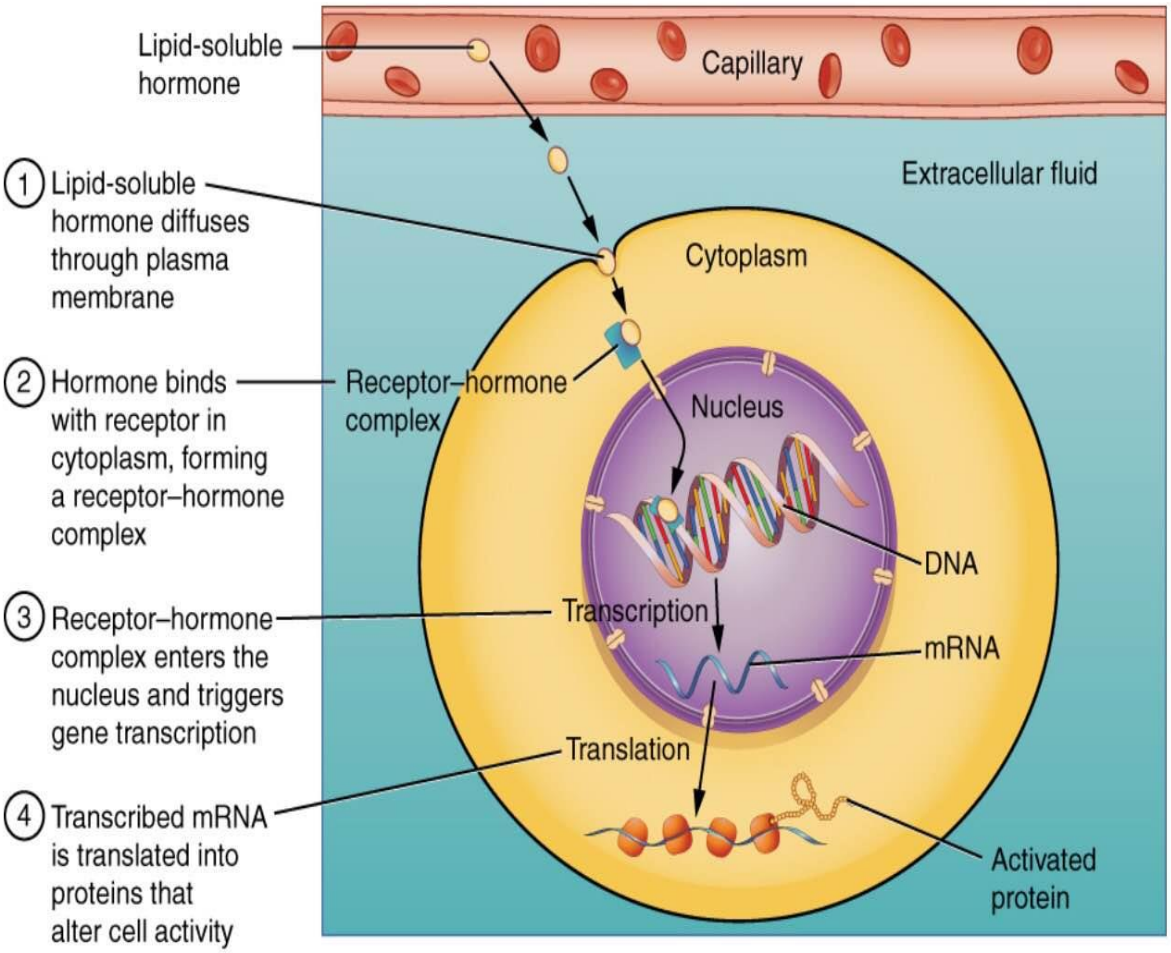
Figure 2: Circulating hormones (endocrine) and local hormones (paracrine and autocrine), taken from [2]

Mechanism of action of Hormones:

Mechanism of hormones` action is mediated by their receptors. These receptors are classified according to their locations and are intracellular receptors or cell surface receptors. Intracellular receptors are located inside the cell and specific for lipid-soluble hormones where they are able to cross the cell membrane. Formation of hormone- receptor complex results in gene expression and affects the physiological responses (Fig. 3A). Cell surface receptors are located on the cell membrane of the target cell and they are either G- protein coupled receptors or enzyme- linked hormone receptors and are specific for water soluble hormones. Activation of those receptors by the binding of the hormone results in activation of many signaling

pathways. An example- includes Gs, Gi, and Gq which are for the G- protein coupled receptors which affect intracellular enzymes such as adenylyl cyclase (AC) and phospholipase C (PLC) or ion channels which in turn affect physiological responses (Fig. 3B). In addition, activation of enzyme- linked hormone receptors leads to activation of some other signaling pathways such as the JAK- STAT pathway which in turn activates intracellular enzymes and induces the physiological effects within the target tissue [4].

A



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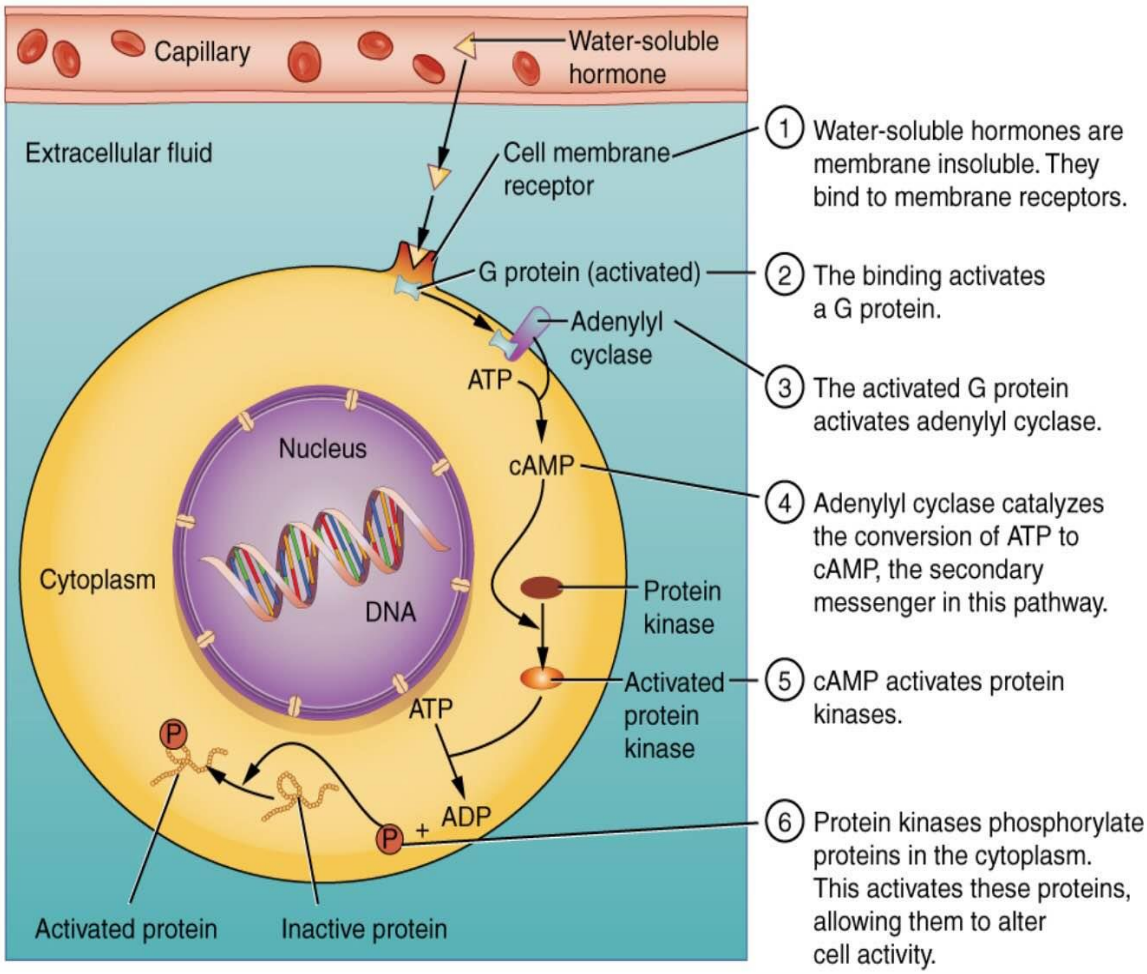


Figure 3: Mechanism of action of hormones. (A) Lipid soluble hormones cross the cell membrane and form hormone- receptor complex to trigger gene expression and physiological response, (B) Water soluble hormones bind to cell surface receptor and activate intracellular enzymes via second messenger to trigger the physiological response, taken from [5].

One of the most important glands is parathyroid gland. Parathyroid glands are four tiny glands located in the neck where they are distributed as two glands on each side of the posterior surface of the thyroid gland. Their size is about 6 mm long, 3-4 mm wide, and 1-2 mm anteroposteriorly. Each gland weighs about 0.04 g. They are classified into superior and inferior parathyroid glands. The third and fourth pharyngeal pouch is responsible for deriving the two parts of parathyroid gland, where the inferior part is derived from the third pouch and the superior part from the

fourth pouch [6]. The parathyroid glands contain two types of cells which are chief cells and oxyphil cells (Fig. 4) [2]. The parathyroid glands play an important role on the whole body especially on bone and kidney by producing parathyroid hormone [6].

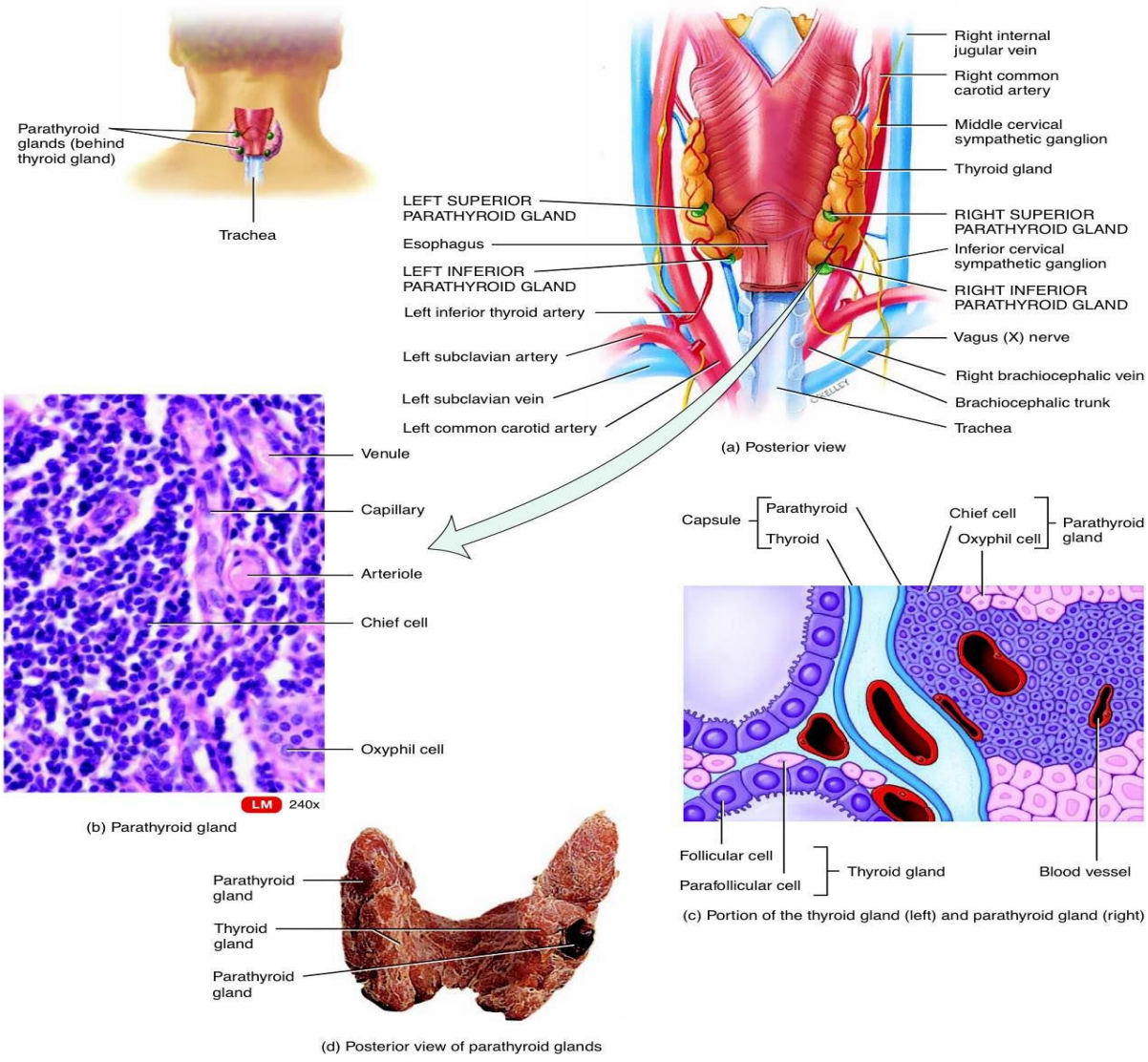


Figure 4: Parathyroid glands, taken from [2]

Parathyroid Hormone (PTH)

Parathyroid hormone is a polypeptide that consists of 84 amino acids produced by parathyroid glands by their chief cells. The main function of PTH is to regulate calcium homeostasis through the body where the chief cells in the parathyroid glands are highly sensitive to the changes in the extracellular calcium levels and produce PTH to increase calcium concentrations or inhibit PTH secretion and then ultimately reduce calcium levels [7]. The parathyroid cells have calcium- sensing receptors (CaSR) on their cell membrane and these receptors are responsible for stimulation of the parathyroid gland to increase or decrease PTH secretion depending on the calcium concentrations (Fig. 5) [8].

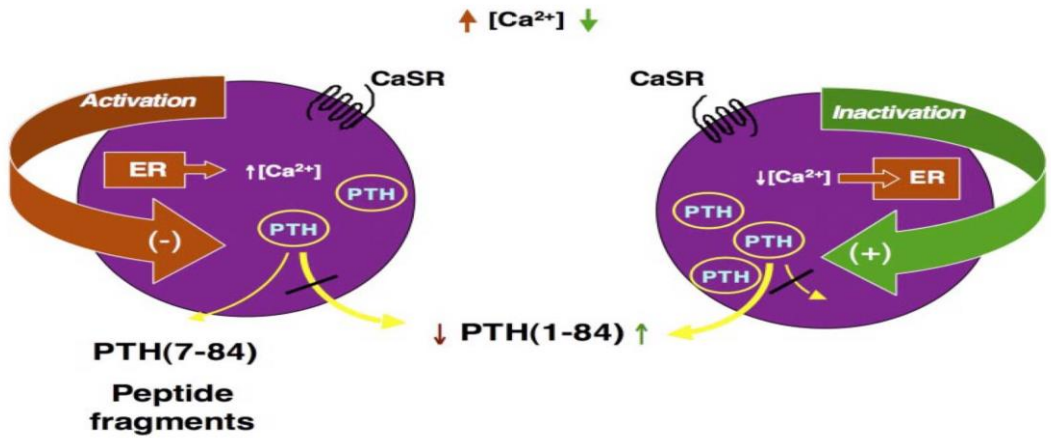


Figure 5: Effect of PTH on calcium levels by CaSR, taken from [9]

Mechanism of action of parathyroid hormone:

Parathyroid hormone action is mediated by the PTH- receptor which is a G- protein coupled receptor located on the cell membrane of the target cell. Activation of this receptor by PTH binding results in activation of many signaling pathways including

Gs and Gq. G- stimulatory (Gs) is linked to adenylyl cyclase (AC), cAMP, and PKA which induce gene expression and catalyze reactions that produce physiological responses. Gq is linked to phospholipase C (PLC), IP3, DAG, and PKC resulting in production of physiological effects (Fig. 6) [10].

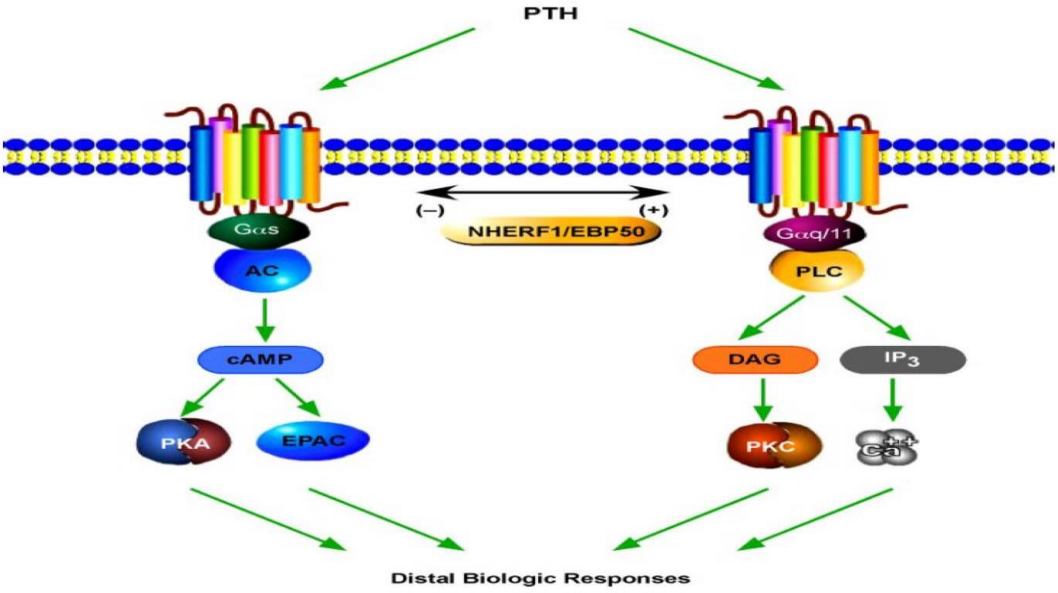


Figure 6: Mechanism of action of PTH, taken from [9].

The principal organs that are directly affected by PTH are the kidney and bone, and there is indirect effect of PTH on the intestine due to activity by 25OH Vitamin D (Fig. 7) [7, 8]. In the kidney, PTH induces reabsorption of renal calcium leading to increase of extracellular calcium levels. This mechanism is regulated by negative feedback as PTH secretion is decreased in the setting of a small elevation in calcium concentration [8], where increase of calcium levels cause degradation of PTH [11]. In addition, PTH is responsible for conversion of 25OH Vitamin D to its active form in the kidney [8]. On the other hand, increasing of parathyroid hormone gene

transcription occurs in response to hypocalcemia [8]. Parathyroid hormone has anabolic and catabolic effects on bone. It can increase bone formation rate and increase bone mineral density (BMD), or it can induce bone resorption to increase extracellular calcium levels as bone is the main storage for calcium [12].

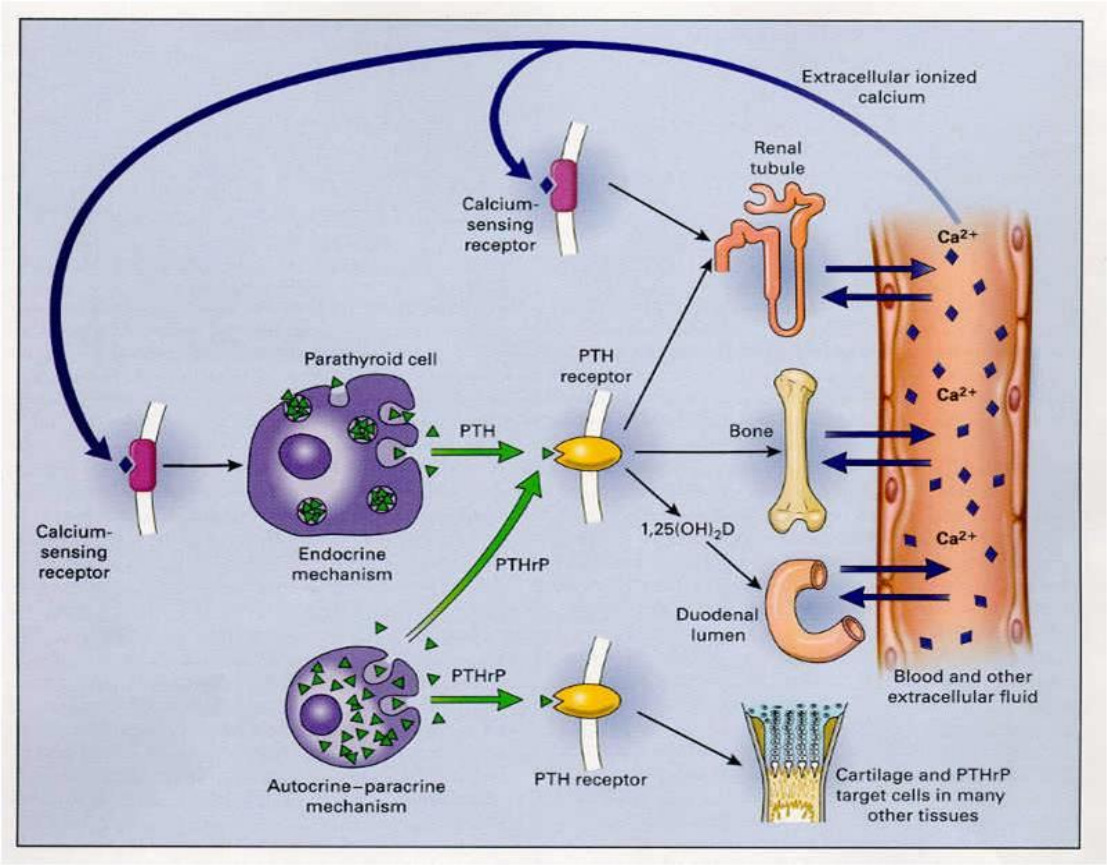


Figure 7: The effects of PTH on different organs, taken from [13]

The anabolic effect of PTH on bone is mediated by activation of PTH/PT- related hormone receptor which is expressed mainly in both bone and kidney [14]. In bone, this receptor is expressed in osteoblast cells lineage, especially in osteocytes, which are differentiated from osteoblasts and compromise about 90- 95 % of mature bone cells [15]. Activation of this receptor by PTH results in reduced expression of sclerostin which is encoded by SOST gene and thereby increases osteoblast activity

and bone formation [14]. At the same time, the catabolic effect of PTH on bone also occurs via activation of PTH/PTrH receptor, which in turn induces osteoclast activity by the expression of RANKL resulting in bone resorption (Fig. 8) [16].

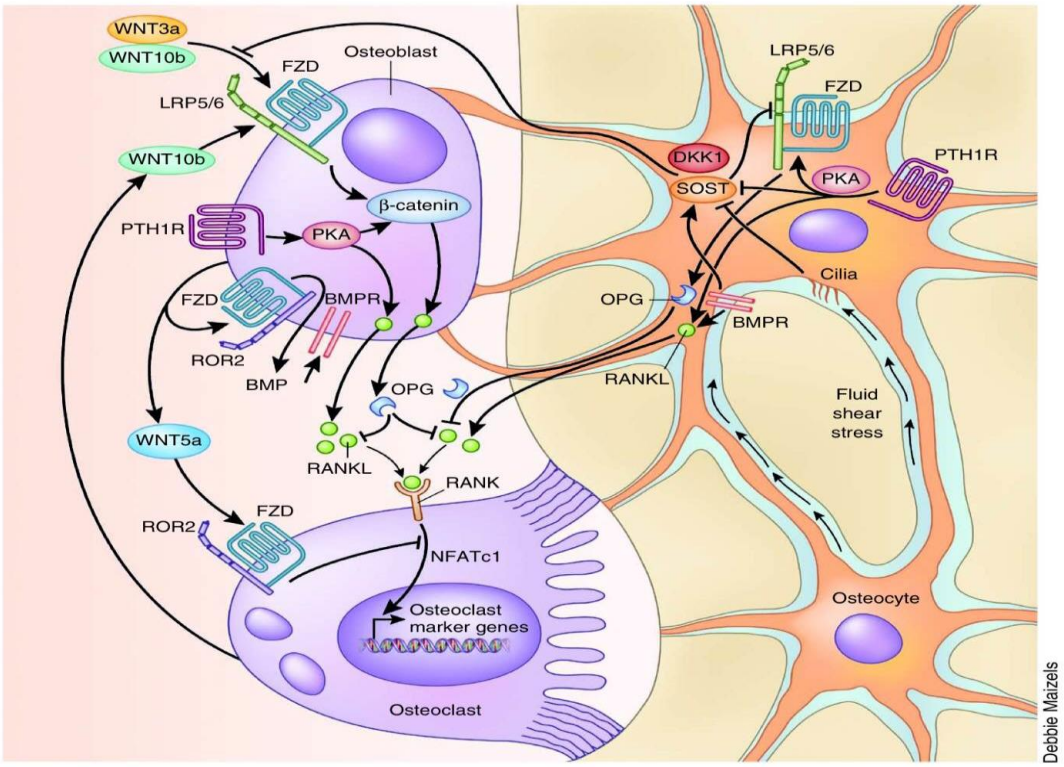


Figure 8: The anabolic and catabolic activity effects of PTH on bone, taken from [17].

The third organ that is affected indirectly by parathyroid hormone is the intestine. PTH as a result of hypocalcemia converts 25OH D to its active form [1, 25 (OH)2 D] via 1 α- hydroxylase enzyme in the proximal cells of the kidney. This occurs due to gene transcription of the 1 α- hydroxylase enzyme gene, CYP27B, by PTH stimulation [18]. Since vitamin D is the regulator of calcium absorption in the intestine [19], PTH has indirect effect on the gut. Hypersecretion of PTH is the presence of high levels of PTH in the blood and cause hyperparathyroidism which is

classified into primary, secondary, and tertiary [20]. Primary hyperparathyroidism (PHPT) results from the autonomous overproduction of PTH or might be a result for abnormal excess in PTH levels [21]. This disorder is caused 80- 85 % by a single adenoma [22]. PHPT is one of the main disorders that affect the parathyroid gland and is considered as calcium metabolism disorder since this disease is characterized by hypercalcemia [23]. In addition, PHPT can cause many metabolic disorders such as dyslipidemia, cardiovascular abnormalities, impaired insulin sensitivity, and glucose tolerance, bone metabolism abnormalities, and obesity [24]. Weakness, depression, and anxiety are clinical presentations in patients with PHPT, in addition fatigability and consciousness impairment might be observed [25]. The medications that contribute in reduction of calcium concentrations or reduce bone mineral density are the main options available for PHPT treatment since there is not a known medical therapy to treat this disease; therefore, parathyroidectomy is recommended for patients with PHPT especially when they have calcium levels more than 1.0 mg/dl above the upper limit and creatinine clearance reduced to less than 60 ml/min [24].

Parathyroid hormone is associated with different biochemical markers which could be affected by increase or decrease of PTH levels such as calcium, phosphors, adenoma weight, 25OH Vitamin D, creatinine, and 24 hr. urinary calcium. In addition, it is associated with different disorders such as dyslipidemia, obesity, cardiovascular diseases, bone abnormalities, and diabetes mellitus.

1.1: Calcium

The main role of PTH is to induce bone resorption, reabsorption of renal calcium, and an indirect effect to increase intestinal calcium absorption, resulting in an elevation of the extracellular calcium levels; therefore, PHPT where PTH levels are overproduced causes hypercalcemia [8]. High concentrations of calcium is considered the first biochemical test to diagnose PHPT and its elevation more than 1.0 mg/dl above the upper limit of normal range is one of the limitations for parathyroidectomy in asymptomatic PHPT [6]. In addition, previous studies have revealed there is a high association between PHPT and hypercalcemia. This hypercalcemia is involved with PHPT in some disorders such as cardiovascular risks. PHPT affects cardiac myocytes by increasing receptor mediated calcium; thereby, calcium enters into myocytes and moves from the sarcoplasmic reticulum leading to damage of myocytes [26]. Moreover, chronic hypercalcemia leads to acceleration of calcium deposition in the coronary arteries and myocardial fibers, and those findings illustrate the role of PHPT in impairment of endothelium. PTH results in- independent vasodilation [27], where PTH is known as a vasodilator by the action of PTH-receptor which is localized on the cells of vascular smooth muscle which acts to induce the elevation of intracellular cyclic adenosine monophosphate (cAMP) [28]. On the other hand, hypercalcemia as a result for PHPT contributes in increasing incidence of hypertension [29]. Depending on the negative feedback mechanism, calcium supposes to reduce PTH secretion and release, but high concentration might affect CaSR on parathyroid adenoma [30].

1.2: Lipid panel

Several studies have described that PHPT is associated with an altered lipid panel and affect the lipid metabolism resulting in dyslipidemia [31]. Lipid abnormalities which are caused by asymptomatic PHPT induce increasing of triglycerides (TG) levels and decreased high-density lipoprotein (HDL) [32], where PTH levels is correlated positively with TG and negatively with HDL [33]. As a result for increased intracellular calcium in the adipocytes by PTH, the activity of adipocytes increase; therefore, there is an increase in the fatty acid synthetase expression, lipogenesis, and reduction in lipolysis [31]. These findings explain the elevation of TG levels and reduction in HDL concentrations.

1.3: Adenoma Weight

One of the variables which could be affected by PHPT is parathyroid adenoma weight. The normal size of parathyroid gland is about 6 mm and its weight is less than 60 mg [34]. The biochemical markers of PHPT may contribute to assist the surgeon in prediction the abnormal tissue of parathyroid gland which supposes to be resected [22]. Some studies demonstrated that biochemical markers and adenoma weight are not significantly correlated, but depending on the research which was performed by Bindlish and his group, they have discovered different results where they have revealed there was a significant positive correlation between adenoma weight with PTH and calcium levels. They have explained the difference between their results and previous studies. The difference might be because they excluded the patients with renal failure and primary parathyroid hyperplasia where PTH secretion rate by cells of

tissues which exhibit renal failure and primary hyperplasia is higher than that by cells from adenomas [35]. Now, it is well described that adenoma weight is positively correlated with PTH and calcium levels.

1.4: BMI

Body Mass index (BMI) is a value derived from the weight divided by squared value of height for an individual (kg/m²). BMI is used for determine if the person is underweight, normal weight, overweight, or obese by quantity the tissue mass amount. It is one of the markers that is associated with PHPT as several studies have confirmed that association. It is well known that patients with PHPT might have high BMI as different studies proved there is a positive correlation between PTH levels and BMI [36]. This relationship between BMI and PHPT is a result for inhibited lipolysis due to increased levels of intracellular calcium in adipocytes [37] since it is known that PTH acts in several cell types to increase the intracellular calcium [38]. Increased BMI might be contributed to increase risks of several diseases such as diabetes mellitus, hypertension, and heart disease by varying proportions [39]. BMI is also associated with increasing risk factor of lipid dysfunction [40] and glucose intolerance [41], and this relationship between BMI and those diseases may provide an explanation for the metabolic abnormalities and increased cardiovascular risks which might be observed in patients with PHPT. The inflammatory changes in the adipose tissue of patients with PHPT illustrate the association between BMI and PTH levels since it is well described that inflammatory genes expression in adipose tissue is increased in obese individuals [24]. As a result for high association between BMI and PTH levels in PHPT, some scientists classified obesity as one of the clinical features

of PHPT [42]. Moreover, vitamin D is negatively correlated with BMI and that explains the hypothesis which refers to increase the risk of vitamin D deficiency; thereby, developing of PHPT [43]. In addition, increased BMI is also correlated with the heavier parathyroid gland weight [44].

1.5: 25OH Vitamin D

25OH Vitamin D is a prehormone that is produced in the liver by hydroxylation of vitamin D3, and also known as calcifediol. It is one of the biochemical markers which is affected by PTH since PTH converts it into the active form of vitamin D (calcitriol) in the kidney to contribute in calcium homeostasis by increasing intestinal calcium absorption [45]. Due to its interaction, it is supposed to be tested in every patient who is suspected to have PHPT [46]. Several studies confirmed that 25OH vitamin D deficiency is inversely associated with PTH levels in patients with PHPT [47], and it is also well described that 25OH D has inverse relationship with adenoma weight of parathyroid gland [48, 49, 50]. The conversion of 25OH D into its active form, calcitriol, is the most common reason for reduction of 25OH D levels in PHPT patients since PTH is responsible for increasing activity of 1 α hydroxylase enzyme to convert 25OH D into 1, 25 (OH)2 D3, calcitriol, in the proximal cells of kidney`s nephrons. At the same time, this conversion of 25OH D into 1, 25 (OH)2 D3 may reduce the dermal synthesis of 25OH D in the liver [51], and the half- life of 25OH D in PHPT patients is shortened as a result for reduced synthesis of 25OH D in the liver [46].

1.6: 24 hr. Urinary Calcium

24 hr. urinary calcium measures the amount of excreted calcium in the urine. The presence of calcium sensing receptor (CaSR) in the kidney is the main factor that mediates excretion of urinary calcium. In PHPT and as a result for hypercalcemia, the urinary calcium excretion is elevated; therefore, it is considered one of the biochemical evaluations for detection and diagnosis of PHPT. In addition, 24 hr. urinary calcium is used for recognizing primary hyperparathyroidism from familial hypocalciuric hypercalcemia (FHH) [52], where FHH is characterized by decreasing urinary calcium levels under 100 mg/L [23] due to a mutation that suppresses the CaSR function [53, 54]. Elevation of 24 hr. urinary calcium levels is a criterion for parathyroidectomy only in the presence of nephrolithiasis or kidney stones [55]; however, some surgeons still look at urinary calcium levels higher than 400 mg/24 hr as an indicator for surgery even in the absence of kidney stones or nephrolithiasis [56].

1.7: Glucose

PHPT can affect glucose metabolism causing hyperglycemia and increased insulin resistance [57]. Many studies have reported that insulin resistance and diabetes mellitus are elevated among PHPT patients [58], in addition to impairment of glucose tolerance [25]. Insulin resistance is increased in PHPT as a result for increased levels of intracellular calcium in adipocytes [58] causing glucose metabolism abnormalities.

1.8: Blood Pressure

The risk of morbidity and mortality in patients with PHPT is mainly represented through cardiovascular diseases [46, 59], and associated with higher elevation in calcium levels [60]. One of those cardiovascular disorders is hypertension [33] and diastolic abnormalities [59] where it was found that hypertension is positively correlated with PTH and calcium levels. The induction of hypertension by PTH might be through PTH- receptor which is located in the myocardium and vessel walls [61]. As a result of direct or indirect interactions of this PTH- receptor, vascular stiffness and remodeling occur causing atherogenesis. Moreover, hypertrophy of the left ventricular is also caused by PTH- receptor interactions in PHPT patients [27, 62, 63].

1.9: Alkaline Phosphatase

Another characteristic factor for PHPT patients is alkaline phosphatase (ALP) since it is one of the bone formation markers [64], and bone formation is highly associated with PTH levels.

1.10: Serum Creatinine

Serum creatinine is also required criteria for PHPT diagnosis to evaluate the renal function in those patients where the highly reduction in creatinine clearance (≥ 30 %) is one of the parathyroidectomy criteria along with 24 hr. urinary calcium and serum calcium levels elevation [23, 65]. PTH reduces reabsorption of bicarbonate in the proximal tubule of the kidney resulting in metabolic acidosis [66]; thereby, the

disruption of the acid – base balance leads to renal failure which is characterized by elevation of serum creatinine levels [67, 68, 69].

1.11: Serum Albumin

Different studies have shown that PTH could affect serum albumin levels by its action on the proximal tubules in the kidney which is result in metabolic acidosis. Metabolic acidosis could reduce serum albumin levels by albumin synthesis impairment [69].

Chapter 2

Background

This study is the first kind to systemically compare the clinical presentations of PHPT patients among different iPTH levels. Primary hyperparathyroidism (PHPT) has become a commonly recognized endocrine disease and is usually caused by a single parathyroid adenoma [70]. The unsuppressed parathyroid hormone (PTH) induces elevated calcium levels and causes clinical presentations ranging from asymptomatic to classic symptoms. Approximately 20 % of PHPT are classified as symptomatic which is characterized by kidney stones, nephrolithiasis, psychiatric abnormalities, overt bone disease, and neuromuscular weakness, and there is not controversy about patients with symptomatic PHPT have to have parathyroidectomy [71]. PHPT who do not have clear symptoms a long with elevation of PTH and calcium levels are described as asymptomatic disease which is most common than symptomatic disorder [72], and it is very frequent among United States and Europe populations which are using multichannel screening tests, while most of symptomatic PHPT patients are among the other countries that have limited using of multichannel screening tests such as China, India, and Brazil [70]. Recent studies revealed higher prevalence of obesity [36], hypertension, hyperlipidemia, type 2 diabetes and coronary artery disease (CAD) in PHPT patients [73]. Regarding obesity, the molecular mechanism of these changes might be consequences for increased of glycerol-3- phosphate dehydrogenase activity and increased fatty acid synthetase

expression that result from elevated intracellular calcium by the action of PTH resulting in reduced lipolysis [31]; thereby, the increased activity of these enzymes leads to increase the volume of adipocyte and increased lipogenesis which in turn rise the triglyceride levels [74]. Additionally, insulin resistance could be affected indirectly by elevated PTH. High levels of PTH in obese individuals stimulate adipose tissue to release more fatty acids than normal individuals; therefore, fatty acids disrupt insulin signaling pathway [75]. Moreover, these released fatty acids contribute in coagulation disorders, myocardial impairment, endothelial defect, and accelerated atherosclerosis [76]. As a result for high prevalence of obesity in PHPT patients, obesity is considered one of the most frequent causes of cardiovascular abnormalities. Previous studies showed that iPTH levels correlated with adenoma weight and serum calcium levels [77], suggesting that iPTH level is likely associated with the severity of disease in PHPT patients. Besides its effects on bone metabolism, iPTH level is also associated with hypertension and heart failure in elderly PHPT [78, 79]. However, there are very few studies comparing the clinical presentations and lipid panel in PHPT patients with different iPTH levels. Hughes *et al* demonstrated that the severity of primary hyperparathyroidism (PHPT) was associated with higher levels of serum calcium in consistent with 25OHD deficiency, single and larger parathyroid adenoma weight [80]. In 2007, Silverberg found that PHPT patient who had higher PTH levels tend to have lowest concentrations of 25OHD. Moreover, patients with lowest 25OHD had lower bone mineral density (BMD), and higher levels of serum ALP [47]. Additionally, Moosgaard *et al* reported that 25OHD deficiency is related to the severity of PHPT where reduced 25OHD might either induces adenoma cells growth through suppression of (MEN1), tumorous suppressor gene, under action of vitamin D receptor (VDR) or rises the parathyroid tissue volume via VDR action also [81]. This

mechanism might be an explanation for association of 25OHD deficiency with the severity of PHPT. We hypothesized that iPTH level affects phenotypes of PHPT patients, which independent of calcium and vitamin D levels. We therefore compared the initial presentations and metabolic characteristics among PHPT patients with different initial iPTH levels.

The aim of this study is to evaluate and compare the differences of characteristic and metabolic features of PHPT between mild iPTH (m-iPTH) and higher iPTH (h-iPTH) levels in patients with PHPT.

Chapter 3

Patients and Methods

212 patients with PHPT were presented at Robert Wood Johnson hospital and assessed for metabolic and characteristic features of PHPT. The initial diagnosis of PHPT was based on: 1) elevated serum intact PTH (iPTH ≥ 65 pg/ml), confirmed hypercalcemia (calcium ≥10.4 mg/dl), and 3) 24 hour urinary calcium > 100 mg/24 hour. Exclusion criteria include familial hypocalciuric hypercalcemia (urinary calcium < 100 mg/24 hour), secondary hyperparathyroidism, patients on lipid medications (above 50 mg daily), patients under vitamin D medications (above 1000Ul daily), renal failure, and multiple endocrine neoplasia. Patients were divided into two groups according to their serum intact parathyroid hormone (iPTH) concentrations. Age, gender, body mass index (BMI), systolic and diastolic blood pressures, fasting blood sugar levels, fasting lipid profile, serum calcium, serum creatinine, serum albumin, 25OH vitamin D, iPTH, serum ALP, and 24-hour urine calcium were measured before surgery. For patients who had parathyroidectomy, adenoma weights were measured in grams. Diagnostic criteria for each disease were as follows: hypertension by a blood pressure ≥ 140/90 mmHg or already on medications to treat hypertension, diabetes mellitus by the ADA criteria of fasting glucose > 126 mg/dl or already on medication to treat diabetes, and obesity by a BMI ≥ 30 kg/ m^2.

3.1 Laboratory Analysis and Procedures

Serum total calcium (mg/dl) was measured by automated methods using enzymatic and colorimetric assays (cobas e analyzers). Patient`s serum (3µl) reacts with 30 µl of reagent containing calcium specific polyamine carboxylic acid. Reaction of calcium ions with a calcium specific polyamine carboxylic acid in an alkaline environment results in formation complex, and thereafter EDTA is added to react with this complex. The change in the absorbance is proportional to calcium concentration. Serum iPTH (pg/ml) was tested by electro-chemiluminescence immunoassay (cobas e analyzers). The principle of this assay is depending on two reactions. N terminal fragment of PTH molecule reacts with biotinylated monoclonal anti-PTH antibody, and C- terminal fragment reacts with monoclonal specific antibody labeled with a ruthenium complex. Addition of streptavidin- labeled microplates results in interaction of biotin with streptavidin inducing of chemiluminescent emission and measured by photomultiplier. Serum 25OHD was assessed by cobas automated immunoassay analyzers. This assay is based on formation of complex which is consist of labeled ruthenium VDBP with biotinylated 25OHD; thereby, 25OHD binds into streptavidin coated microplates to be measured by a photomultiplier by induction of chemiluminescent emission via voltage applied to the electrode. Lipid Panel including total cholesterol, triglyceride, high density lipoprotein (HDL), and low density lipoprotein (LDL) were measured by colorimetric and enzymatic methods via cobas e analyzers. The principle of cholesterol and HDL- cholesterol assessments are depending on reactions that result in hydrolyzation of cholesteryl esters by cholesterol esterase, generation of cholestenone and NADH by cholesterol dehydrogenase, and formation of formazan dye by diaphorase enzyme through oxidation- reduction

reaction. The intensity of formazan dye is proportional to cholesterol and HDL-cholesterol concentrations. Similar principle for TG measurement by the hydrolyzation of TG into glycerol and fatty acids by lipoprotein lipase enzyme. Formation of NADH by glycerol and NAD+ reaction in the presence of glycerol dehydrogenase, and then formazan dye is formed through oxidation- reduction reaction by diaphorase enzyme. The color intensity of formazan dye is proportional to TG concentration. LDL was calculated by Friedewald formula:

LDL= T. cholesterol - HDL - (TG\5)

Serum glucose (mg/dl) was measured by colorimetric and enzymatic assay (cobas e analyzers). Reaction of glucose with ATP and oxidation of G-6-P by G6PD enzyme to form 6-phosphoglucanate and NADH. NADH concentration is proportional to glucose concentration (Enzymatic methods of analysis). Alkaline phosphatase (Ul/L) is also tested by colorimetric assay (cobas e analyzers). In this assay, p-nitrophenyle phosphates were cleaved into phosphate and p-nitrophenol by phosphatases. Serum albumin was measured by cobas e analyzers depending on colorimetric assay where binding of albumin into bromocerol green (BCG) results in the induction of color to measure albumin concentration. Serum creatinine was tested by colorimetric method via cobas e analyzers. In this assay, serum creatinine principle is depending on conversion of creatinine and release of hydrogen peroxide (H2O2) and in the presence of peroxidase enzyme which induces colored product. BMI was calculated by an equation which is compromised weight and height of the patient. Body`s weight in kilograms was divided by the square of the body`s height in meters.

24 hour urinary calcium was performed by laboratory techniques. 24 hour urine was collected in a plastic container acidified by addition of 6 mol/L of HCL to each 3 ml of urine. Reaction of calcium with 5-nitro-5`methyle-BAPTA (NM-BAPTA) in an alkaline environment forms a complex. Addition of EDTA to this reaction results in formation of two complexes and the change in the absorbance is proportional to urinary calcium concentration.

3.2 Statistical Analysis

Results are expressed as mean ± SD and the number of patients in each category and percentage of total patients in that group. Analysis of variance (ANOVA) and Tukey`s multiple comparison post hoc tests were used to compare means among groups. The prevalence rates between groups were compared with chi-square tests for significance. Correlation coefficients and linear regression were used to assess relationships. Two sided P value < 0.05 were selected as the level of significance.

Chapter 4

Results

4.1: Correlation of biochemical markers with iPTH levels

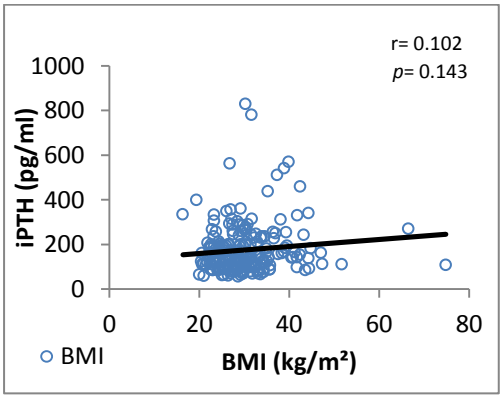
Higher levels of iPTH in patients with PHPT are associated with many biochemical markers as previous studies have shown that. To confirm these correlations, we focused on correlations of characteristic and metabolic features of PHPT with iPTH levels. The correlation of iPTH with biochemical markers is shown on table 1. We found that body mass index (BMI) was not correlated with iPTH levels ($r = 0.102$, $p > 0.05$), as well as serum creatinine was not correlated with iPTH levels ($r = 0.1$, $p = 0.3$) [Fig. 9A, D]. In addition, calcium and adenoma weight had significant positive correlation with elevated levels of iPTH in PHPT patients ($r = 0.297$, $p < 0.001$; $r = 0.472$, $p = 0.00001$) respectively [Fig. 9B, C]. Furthermore, triglycerides were positively correlated with iPTH levels ($r = 0.141$, $p = 0.04$), but there was a negative correlation between high density lipoprotein (HDL) and iPTH levels ($r = - 0.149$, $p = 0.03$). At the same time, we did not find any correlation between T- cholesterol and iPTH levels [Fig. 10A, B, and C]. Moreover, 25OH D had a negative correlation with iPTH concentrations in PHPT patients ($r = - 0.19$, $p = 0.01$) [Fig. 10D]. We also found that 24 hr urinary calcium and alkaline phosphatase were positively correlated with iPTH levels, but they were not significant ($r = 0.127$, $p = 0.09$; $r = 0.168$, $p = 0.08$). Additionally, serum albumin was not correlated with iPTH concentrations in patients with PHPT($r = - 0.08$, $p = 0.4$). On the other hand, we did not observe any correlation between serum glucose, SBP, and DBP with iPTH levels.

Item	Correlation	*P*. value
Age (yo)	0.02	0.77
BMI (Kg/m^2)	0.102	0.143
Calcium (mg/dl)	0.297	< 0.001
TG (mg/dl)	0.141	0.04
HDL (mg/dl)	-0.149	0.03
25OH VIT (ng/ml)	-0.19	0.01
Ad. Weight (g)	0.472	< 0.001
Glucose (mg/dl)	-0.032	0.76
SBP (mmHg)	0.061	0.38
DBP (mmHg)	-0.021	0.76
24hr Calcium (mg/24hr)	0.127	0.09
Creatinine (mg/dl)	0.1	0.3
Albumin (g/dl)	-0.08	0.4
ALP (Ul/L)	0.168	0.08

Table.1: Correlation of characteristic and metabolic features of PHPT with iPTH levels in patients with PHPT disorder.

Abbreviations: BMI= Body Mass Index; TG= Triglycerides; HDL= High Density Lipoprotein; SBP= Systolic Blood Pressure; ALP= Alkaline Phosphatase

A



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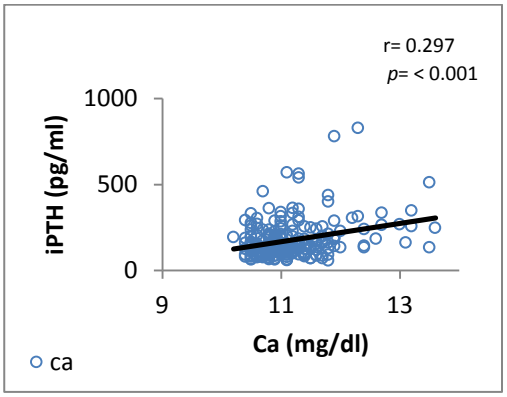

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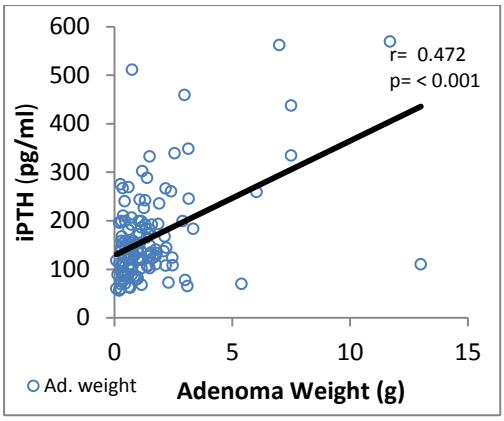

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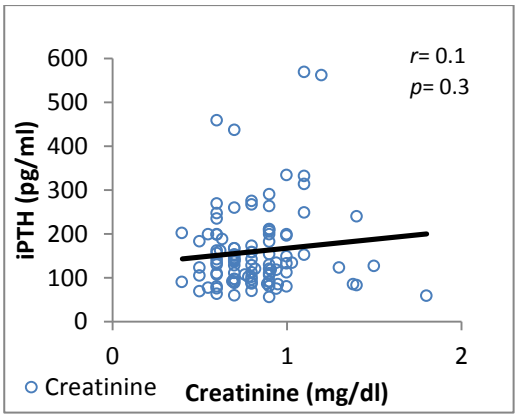
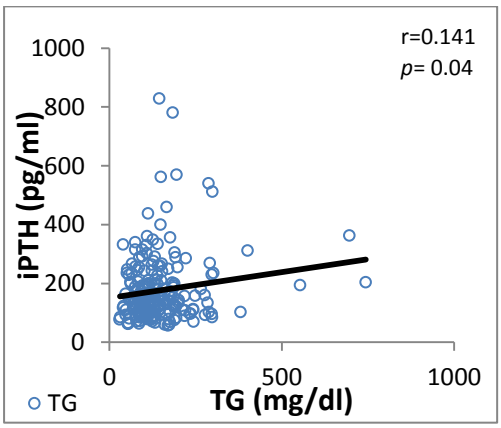


Figure 9: Correlation of characteristic features of PHPT with iPTH levels. (A) represents the correlation between BMI and iPTH (not significant: r= 0.102, p > 0.05), (B) shows the positive correlation of calcium with iPTH (r=0.297, p < 0.001), (C) refers to the positive correlation between adenoma weight and iPTH (r= 0.47, p < 0.001), and (D) shows there is no correlation between serum creatinine with iPTH levels (r= 0.1, p= 0.3). [r= correlation coefficient, p= the significant level].

A



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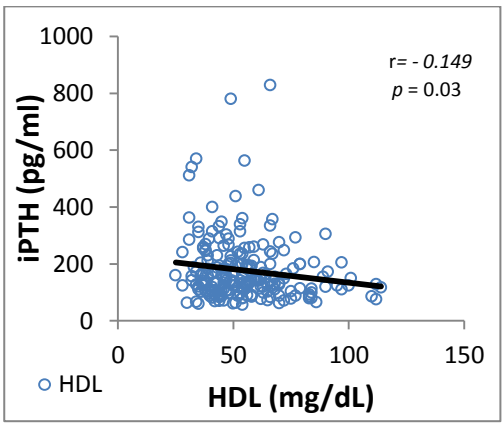


C D

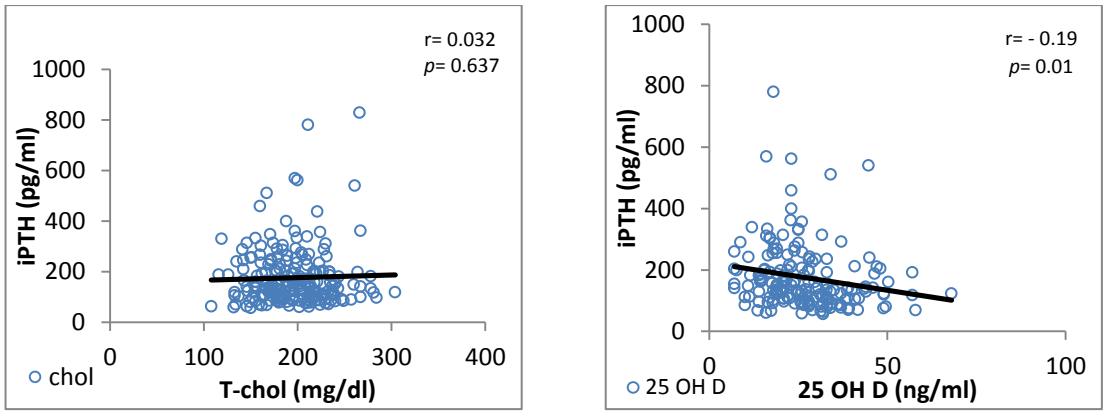


Figure 10: Correlation of Lipid panel and 25OH D Vitamin with iPTH levels in patients with PHPT. (A) shows the positive correlation between TG and iPTH (*r*= 0.141, *p*= 0.04), (B) refers to the negative correlation of HDL with iPTH levels (*r*= - 0.149, *p*= 0.03), (C) shows there is no correlation between T- cholesterol and iPTH levels (*r*= 0.03, *p*= 0.637), and (D) represents the negative correlation between 25OH D Vitamin and iPTH levels (*r*= - 0.19, *p*= 0.01). [*r*= correlation coefficient, *p*= the significant level].

4.2: Comparison of Characteristic features between m-iPTH and h-iPTH levels

To find out the effects of different levels of iPTH in patients with PHPT on the body, we divided the subjects into two groups depending on iPTH levels (mild and higher). Mild- iPTH refers to iPTH concentrations below than 140 (pg/ml), whereas higher-iPTH represents iPTH levels above 140 (pg/ml).

Of the 212 PHPT patients, 100 (17 males and 83 females) who had serum iPTH below 140 pg/ml (102 ± 22.6) were classified as m-iPTH group, whereas 112 (25 males and 87 females) with serum iPTH above 140 pg/ml (242.4 ± 118.7) were classified as h-iPTH group as shown in table 2. For characteristic features which include age, iPTH, calcium, 25OH D, ALP, and adenoma weight, h-iPTH patients

were younger than m-iPTH group (58.4 ± 12.9 yo *vs*. 62 ± 11.6 yo, *p*= 0.03) [Fig. 11A]. The h-iPTH patients, compared with m-iPTH patients had significantly higher calcium level (11.2 ± 0.73 mg/dl *vs*. 11.0 ± 0.46 mg/dl, *p*= 0.003) [Fig. 11B], lower 25OH D (24 ± 10.8 ng/ml vs 29.9 ± 11.1 ng/ml, p= 0.0006) [Fig. 11C]. Additionally, we found that h-iPTH patients had significantly higher ALP in comparison with m-iPTH patients (90.8 ± 24.9 Ul/L *vs* 77.7 ± 17.5 Ul/L, *p*= 0.002) [Fig. 11D]. Of the 100 m-iPTH patients, 74 patients had parathyroidectomy and of the 112 h- iPTH patients, 75 patients had parathyroidectomy. The adenoma weight in h- iPTH was 1.70 ± 1.93 gram and 1.14 ± 1.56 gram in m- iPTH group (*p*= 0.06) [Fig. 11E].

	m-PHPT	h-PHPT	P. value
Age Yr	62 ± 11.6	58.4 ± 12.9	0.03
Gender M/F	17/83	25/87	0.21
iPTH pg/ml	102 ± 22.6	242.4 ± 118.7	< 0.001
Calcium mg/dl	11 ± 0.46	11.2 ± 0.73	0.003
25OHD ng/ml	29.9 ± 11.1	24 ± 10.8	0.0006
Ad. Weight G	1.1 ± 1.6	1.7 ± 1.9	0.06
24hr. Ur. Calcium mg/24 hr	304.5 ± 155.3	330.9 ± 160.5	0.291
Creatinine mg/dl	0.83 ± 0.25	0.80 ± 0.2	0.59
Albumin g/dl	4.2 ± 0.45	4.2 ± 0.32	0.85
ALP Ul/L	77.7 ± 17.58	90.8 ± 24.9	0.002

Table.2: Differences of characteristic features of PHPT disorder between m-iPTH and h-iPTH levels

Abbreviations: m-iPTH= Mild intact PTH; h-iPTH = Higher intact PTH

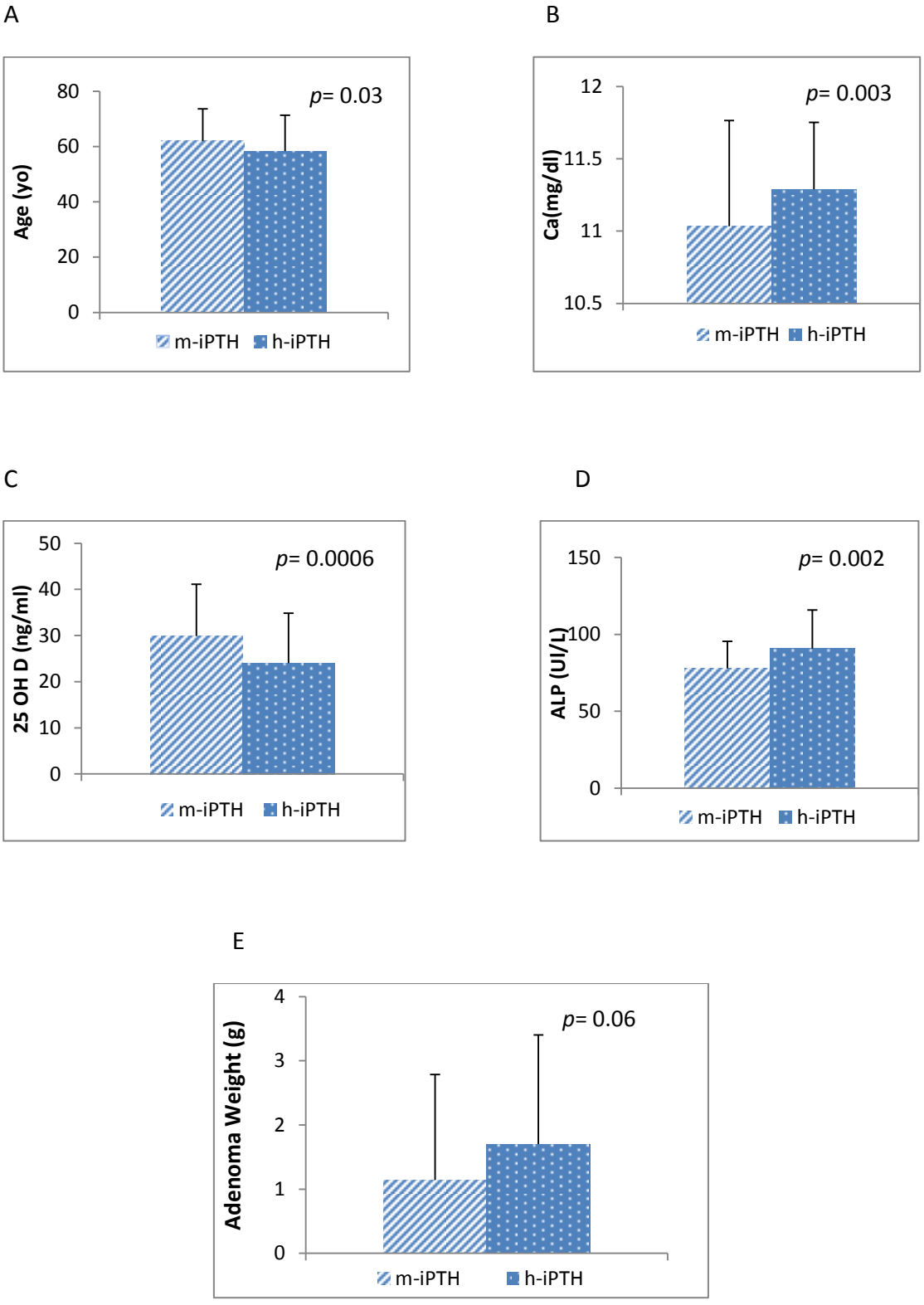


Figure 11: Differences of characteristic features of PHPT between m-iPTH and h-iPTH levels. (A) bar refers to age differences (p= 0.03), (B) bar represents higher calcium levels in h-iPTH when compared with m-iPTH (p= 0.003), (C) bar explains the reduction in 25OH D in h-iPTH patients in comparison with m-iPTH patients (p= 0.0006), (D) bar refers to higher ALP levels in patients with h-iPTH (p= 0.002), and (E) bar explain the differences of adenoma weight between m-iPTH and h-iPTH levels (p= 0.06). Shown is Mean ± SD.

4.3: Comparison of metabolic features between m-iPTH and h-iPTH levels

We focused also on the metabolic features of PHPT including BMI, glucose, T-cholesterol, triglycerides, HDL, SBP, and DBP which is shown in table 3. We found that h-iPTH patinets had a significantly lower HDL in comparison with m-iPTH patients (52.6 ± 15.4 mg/dl vs. 57.8 ± 19.6 mg/dl, p= 0.03) [Fig. 12]. Additionally, there was no significant different in the other metabolic features.

	m-PHPT	h-PHPT	*P*. value
BMI Kg/m²	29.5±7.36	31±7.28	0.147
Glucose mg/dl	109.8±29.2	106.3±18.3	0.479
T- cholesterol mg/dl	197 ± 36.9	194 ± 34	0.65
TG mg/dl	135.8±66.6	152.5±106.9	0.182
HDL mg/dl	57.8±19.6	52.6±15.4	0.03
SBP mmHg	135.9±17.9	134.6±16.6	0.595
DBP mmHg	79.5±9.4	77.6±11.4	0.223
DM %	17 %	24 %	0.3

Table 3: Differences of metabolic features of PHPT between m-iPTH and h- iPTH levels.

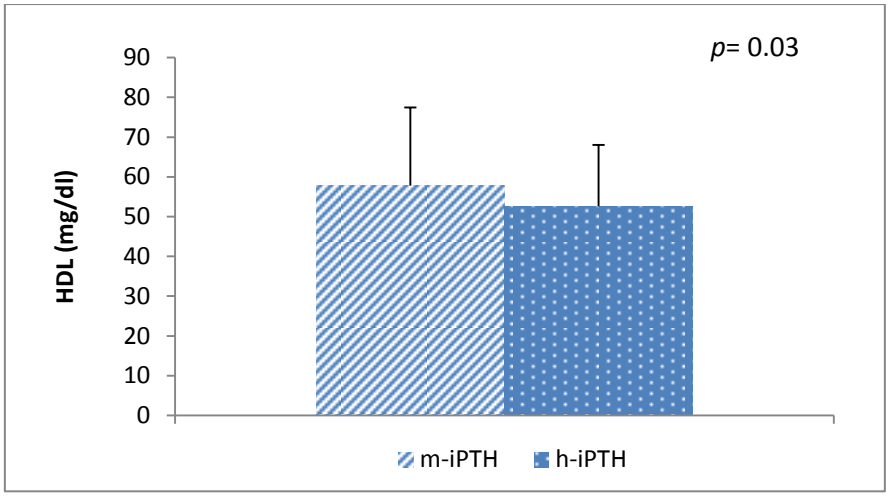


Figure 12: Lowering HDL levels in h-iPTH patients in comparison with m-iPTH patients. Shown is Mean ± SD.

4.4: Comparison of characteristic features between m-iPTH and h-iPTH levels after matching age (40- 85) years old

After matching age and limit the participated ages between 40- 85 years old, 194 patients with PHPT were used as subjects. Of 194 patients with PHPT, 93 (15 males and 78 females) who had iPTH less than 140 pg/ml (m-iPTH), and 101 (23 males and 78 females) had serum iPTH more than 140 pg/ml (h-iPTH). We found that age was different about before matching the age, and there was no significant lowering in age of h-iPTH patients (60.7 ± 10 yo *vs*. 62 ± 11.6 yo, $p > 0.05$) [Fig. 13A]. The other characteristic features which include calcium, 25OHD, ALP, and adenoma weight did not change and still have difference between m-iPTH and h-iPTH even after matching the age as shown in (Table 4).

4.5: Comparison of metabolic features between m-iPTH and h-iPTH levels after matching age (40- 85) years old

Furthermore, the metabolic features did not change after matching the age, and h-iPTH patients still have a significant lower HDL than m-iPTH patients (52.6 ± 15.4 mg/dl vs. 57.8 ± 19 mg/dl, p= 0.04) [Fig. 13B]. In addition, TG and the other metabolic features still do not have difference between m-iPTH and h-iPTH patients (p > 0.05) [Table 5].

	m-iPTH	h-iPTH	*p*. value
Age Yo	62 ± 11.6	60.7 ± 10	0.3
Gender M/F	15/78	23/78	0.13
Calcium mg/dl	11.0 ± 0.47	11.2 ± 0.66	0.01
iPTH pg/ml	102.6 ± 22.3	240.5 ± 120.4	< 0.001
25OH D ng/ml	30.6 ± 11.0	24.3 ± 11.0	0.0004
Ad. Weight G	1.18 ± 1.69	1.7 ± 2.0	0.06
24 hr. calcium mg/24hr	307 ± 159.8	327 ± 161.5	0.44
Creatinine mg/dl	0.81 ± 0.22	0.84 ± 0.31	0.6
Albumin g/dl	4.29 ± 0.46	4.2 ± 0.32	0.29
ALP Ul/L	79 ± 17.1	91.8 ± 25.9	0.005

Table 4: Differences of characteristic features of PHPT between m-iPTH and h-iPTH levels after matching the age (40- 85) years.

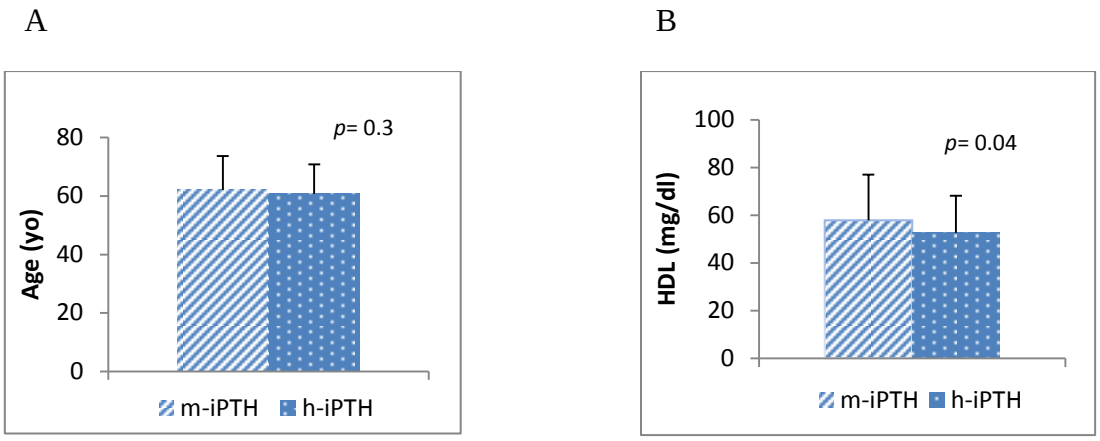


Figure 13: (A) The age status in m- iPTH and h- iPTH groups after matching age (40- 85) years old, (B) HDL status after matching the age. Shown is Mean ±SD.

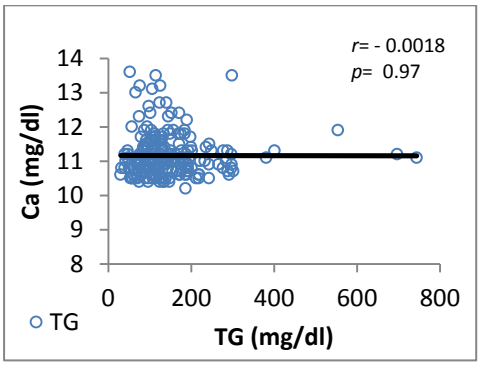
	m-iPTH	h-iPTH	*P*. value
BMI kg/m²	29.5 ± 7.4	30.9 ± 6.5	0.18
Glucose mg/dl	110 ± 29.9	105.5 ± 18.5	0.38
T-cholesterol mg/dl	198.8 ± 37.1	195.5 ± 34.9	0.53
TG mg/dl	132.2 ± 63.4	151.1 ± 108.7	0.14
HDL mg/dl	57.8 ± 19	52.6 ± 15.4	0.04
SBP mmHg	136.8 ± 18.2	135.4 ± 16.3	0.61
DBP mmHg	79.7 ± 9.3	78 ± 11.6	0.28
DM %	19 %	24 %	0.5

Table 5: Differences of metabolic features of PHPT between m-iPTH and h-iPTH after matching the age (40- 85) years.

4.6: Intact PTH levels affect the biochemical markers independent or dependent of serum calcium and 25OHD levels

Moreover, to evaluate whether those correlations with iPTH were independent of calcium and 25OHD levels or not, we analyzed their associations with calcium and 25OHD concentrations. For lipid panel, we found that TG and HDL were not correlated with calcium levels ($p > 0.05$) for both. On the other hand, we observed that only TG was negatively correlated with 25OHD ($r = -0.185$, $p = 0.01$), but there was not any correlation between HDL and 25OHD ($p > 0.05$) [Fig. 14].

A



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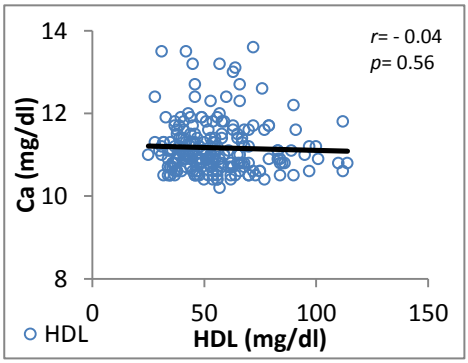

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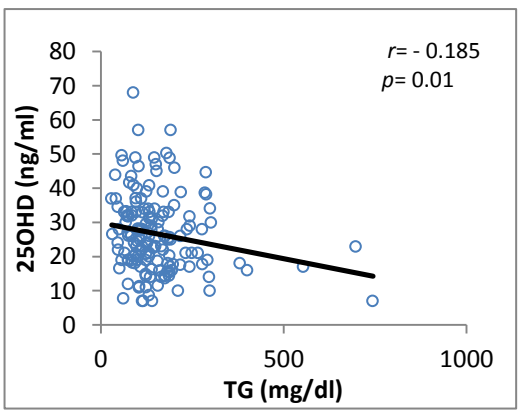

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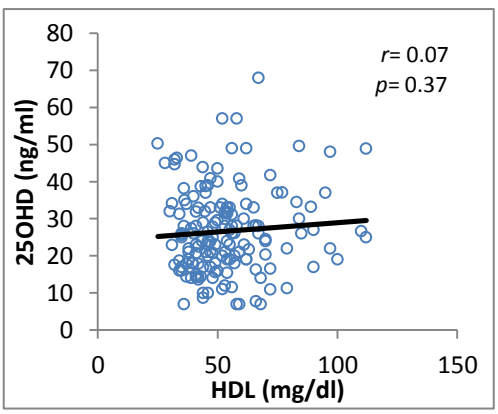

Figure 14: Linear correlation between TG and HDL with calcium and 25OHD levels. (A) shows there is not correlation between hypertriglyceridemia and hypercalcemia, (B) demonstrates that HDL does not have any association with hypercalcemia, (C) represents the positive correlation between TG and 25OHD, and (D) explains that HDL and reduced 25OHD are not correlated.

Thereafter, we evaluated the correlations of adenoma weight with calcium and 25OHD concentrations. We found that adenoma weight was correlated positively with calcium and negatively with 25OHD levels, but it was not significant in both of them (r= 0.135, p= 0.124/ r= - 0.125, p= 0.16) respectively [Fig. 15].

A B

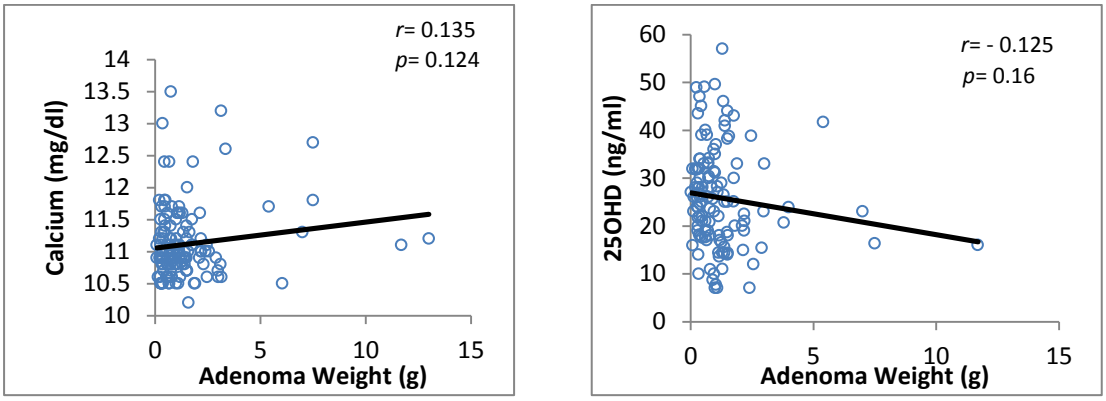

Figure 15: (A) Not significant positive association between parathyroid adenoma weight and calcium levels, while (B) demonstrate parathyroid adenoma weight has an inverse relationship with 25OHD but not significant.

Additionally, we analyzed the other biochemical marker that is serum alkaline phosphatase (ALP) with both serum calcium and 25OHD also. We observed that ALP had a positive association with calcium concentrations (r= 0.26, p= 0.007) respectively, but it did not have any correlation with 25OHD (r= 0.03, p= 0.77) [Fig. 16].

A B

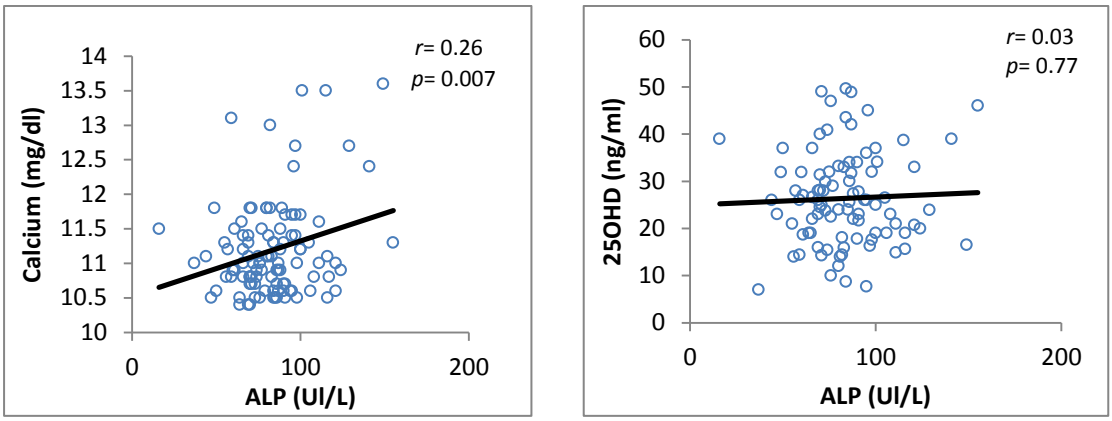

Figure 16: Linear correlation represents the positive association of ALP with serum calcium (A), whereas (B) demonstrates that serum ALP does not associated with reduced 25OHD in patients with PHPT.

Chapter 5

5.1: Discussion

Primary hyperparathyroidism has become a common disorder but the management of PHPT remains controversial [82]. Adenoma weight is one of the major determinants of disease severity and is positively correlated with iPTH levels [83]. The main objective of this study was to compare primary hyperparathyroidism (PHPT) patients with different iPTH levels for serum calcium levels and other laboratory values. In addition, we focused on the correlation of characteristic and metabolic features of PHPT in patients with PHPT to confirm the results of previous studies. Our study is the first of this kind to systemically compare the clinical presentation of PHPT patients among different iPTH levels. Our results confirmed the significant positive association between iPTH levels in PHPT with calcium and adenoma weight (p = < 0.001, < 0.00001) respectively, thus the h-iPTH patient tends to have more severe PHPT characteristics [73, 77]. It is normal for positive association between iPTH and calcium levels since parathyroid hormone is the principal regulator for calcium homeostasis [7]. For adenoma weight, it might be associated positively with iPTH levels as a result of the role of PTH in the reduction of lipolysis through increasing intracellular calcium in the adipocyte, or because of 25OHD deficiency as witnessed in this study. The effect of 25OHD on parathyroid adenoma occurs by vitamin D receptor (VDR) on parathyroid cells, which has an essential role in the inhibition of parathyroid cell proliferation; therefore, 25OHD deficiency induces parathyroid adenoma growth [84, 85]. Additionally, we found that 24 hr. urinary calcium had a positive relationship with iPTH levels, but it was not

significant ($p > 0.05$). This relation might be a result for hypercalcemia via CaSR. We also observed that triglycerides were positively correlated with iPTH levels, and there was a negative correlation between HDL and iPTH concentrations in patients with PHPT. These observations might be a consequence of the action of PTH directly increasing intracellular calcium in adipocytes [86], or indirectly through conversion of 25OHD into D3, calcitriol, where vitamin D3 can contribute in increasing of intracellular calcium levels [87]. Intracellular calcium reduces the phosphorylation of hormone sensitive lipase enzyme, the key of lipolysis, and inhibits adenylyl cyclase and cAMP through β- adrenergic pathway mediated by phosphodiesterase 3B enzyme activation resulting in lipolysis inhibition [88]. These findings might illustrate the slight positive correlation between BMI and iPTH levels in this study despite no significance ($p > 0.05$) since obesity is associated with high levels of triglycerides and lipolysis impairment [89]. We also found that 25OHD had inverse relationship with iPTH levels in PHPT patients ($p = 0.01$). Furthermore and to analyze the main object of this study, we divided our subjects into m-iPTH and h-iPTH patients to compare the differences of characteristic and metabolic features of PHPT between those groups. Our results suggest that h-iPTH patients tend to be 4 years younger than m-iPTH patients. A recent report showed PHPT patients with cystic adenoma were 5 years younger compared with patients with solid adenoma [90]. As aging is associated with metabolic symptoms, differences in more metabolic disorder markers in h- iPTH patients might be present if age was comparable between the two groups. Additionally, we found that PHPT patients had lower HDL levels independent of calcium levels, consistent with previous studies reporting correlation of iPTH levels with metabolic syndrome [91, 92, 93], and we demonstrated that lowered HDL levels is associated with the severity of PHPT. The higher iPTH levels may have an impact

on metabolic outcomes in addition to its effects on mineral and bone metabolism [91]. The group of h-iPTH levels had lower 25OHD in comparison with m-iPTH group. Moreover, h-iPTH had a significant higher serum calcium and ALP. Adenoma weight was higher also in h-iPTH patients, and it was very close to being significant (p = 0.06). While the underlying mechanisms for these changes are unclear, we speculate that elevated iPTH levels might decrease HDL by increasing insulin resistance directly or indirectly by weight gain [94], and the action of increased hepatic lipase enzyme interacted with TG enrichment of HDL that occur during increased insulin resistance to promote catabolism of HDL and decrease in HDL concentrations [95], not through increasing intracellular calcium levels in adipocytes which results in lipolysis inhibition [86, 88] since decreased HDL levels were associated with only high levels of iPTH and were independent of calcium levels as observed in this study. On the other hand, the exact reason for decreased 25OHD along with higher levels of iPTH is not clear, but it may be a consequence of increased conversion of 25OHD into 1, 25 (OH) 2 D3, the active form of vitamin D, in the kidney by PTH, or this conversion may lead to decreasing synthesis of dermal 25OHD in the liver [51]. Additionally, serum ALP was higher in h-iPTH than m-iPTH patients. This increased ALP was either a result for PTH action on bone which is mediated by PTH- receptor expressed in osteoblast cells [15] since ALP was described as an essential bone marker for differentiation of osteoblasts [96], or ALP was increased as a consequence for 25OHD deficiency which results in impairment of bone formation and mineralization [97]; therefore, increased ALP explains increased osteoblastic activity that follows bone resorption [98]. Despite the guidelines for PHPT management having been revised in recent years, the appropriate management of PHPT patients awaits evidence from new clinical studies. The recently revised guideline focuses on

several clinical manifestations including age, serum calcium levels, 24- hour urinary calcium, and the incidence of renal stone and bone disorders [99]. Parathyroid surgery decreases iPTH and calcium levels, and therefore, reduces risks of bone fracture in PHPT patients [100]. Parathyroidectomy may also normalize dyslipidemia [40] and reduce insulin residence [92], which improves structural and functional defects associated with atherosclerosis [101]. We therefore suggest that the guidelines should consider iPTH level as one of the indications for surgery as iPTH levels associate with PHPT severity and metabolic disorder. Further study of a larger cohort is warranted to study whether iPTH levels should be included as recommendation for surgery.

To confirm our hypothesis that suggests iPTH levels affect phenotypes of PHPT patients independent of calcium and 25OHD levels, we focused on markers that are correlated with iPTH or the severity of PHPT disease with both serum calcium and 25OHD. Our results supported our hypothesis for some markers, where we found that the correlation of TG and HDL whether positively or negatively were independent of calcium and 25OHD levels except for TG which had significantly negative correlation with 25OHD. Furthermore, iPTH effects on adenoma weight seem to be independent of calcium and 25OHD levels since the association of parathyroid adenoma weight with both serum calcium and 25OHD was not significant. However, we cannot ignore the role of reduced 25OHD in parathyroid cell proliferation as previous studies confirmed that role and we can observe that clearly in the linear correlation between them (Fig. 12B) despite this correlation not being significant. Additionally, our data demonstrated that the significant correlation of serum ALP with iPTH was close to being significant (p= 0.08). It was dependent of serum calcium levels but independent of 25OHD concentrations where we found ALP did not have any relationship with

25OHD levels. Hence the reason of increased serum ALP in PHPT patients seems to be a result of the direct action of PTH via PTH- receptor, which is expressed in osteoblasts, not a consequence for reduced 25OHD through VDR as other studies have shown.

5.2: Conclusion

Our study is the first of this kind to systemically compare the clinical presentation of PHPT patients among different iPTH levels. In addition to confirmation of different correlations between characteristic and metabolic markers, we demonstrated that HDL levels are associated with severity of PHPT, increased ALP in PHPT patients is a result of the action of PTH not by 25OHD deficiency, and we found a negative correlation between TG and 25OHD. This study supported our hypothesis that iPTH levels affect phenotypes of PHPT patients where we demonstrated that h-iPTH levels are associated with the risks of dyslipidemia independent of calcium and 25OHD levels. Additionally, intact PTH levels are an important factor to contribute in the management of PHPT patients. Intact PTH levels, lipid panel, 25OHD, ALP, in addition to the calcium levels might also need to be considered in the therapeutic decision for PHPT patients.

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