ISSN: 2378-3397

Arrangoiz et al. Int J Surg Res Pract 2018, 5:064
DOI: 10.23937/2378-3397/1410064
Volume 5 | Issue 1
Open Access

International Journal of
Surgery Research and Practice

RESEARCH ARTICLE

Does Vitamin D Deficiency Cause Primary Hyperparathyroidism?

Rodrigo Arrangoiz*, Jorge Sánchez-García, Eduardo Moreno-Paquentin, David Caba Molina, Enrique Luque de León, Fernando Cordera, Manuel Muñoz, Efraín Cruz-González and Luis Fernando Negrete-Cervantes

Department of General Surgery and Surgical Oncology, American British Cowdray Medical Center, Mexico

***Corresponding author:** *Rodrigo Arrangoiz, MS, MD, FACS, Department of General Surgery and Surgical Oncology, Sociedad Quirurgica S.C., American British Cowdray Medical Center, Mexico City, Mexico, Tel: 52-55-1664-7200, E-mail: rodrigo.arrangoiz@gmail.com*

Abstract

Background: Primary hyperparathyroidism (PHPT) is the third most common endocrine disease. It is caused by a single parathyroid adenoma in 85% to 90% of the cases. Vitamin D deficiency (VDD) is a common finding in PHPT with an incidence varying in the literature from 53% to 77%. The aim of our study is to describe a cohort of patients with PHTP with and without VDD.

Methods: Retrospective study from a prospectively kept database of patients with PHPT treated by our group between January 2015 and July 2017. The operation performed was a bilateral neck exploration through a two centimeter incision with radioguided parathyroidectomy. The patients' characteristics were obtained and analysed from the electronic medical records. Patients without complete medical records were not included in our study. All data were collected in a non-identifiable fashion in accordance with the principles outlined in the Declaration of Helsinki and as required for our institutional review board approval.

Results and Discussion: A total of 50 patients with PHPT were included, four fifths were females and one fifth were males; the mean age of the patients in our study was 56.7 years. The mean preoperative PTH was 97 pg/ml with significant decreased postoperative value (all patients were cured, normal calcium and PTH levels 12 months after the operation). The mean preoperative calcium was 10.2 mg/dl. Thirty-five patients (70%) had preoperatively VDD before surgery and had subjective exacerbation of their PHPT symptoms when vitamin D was giving in order to try and correct the deficit. The mean preoperative vitamin D (VD) level was 27.5 ng/dl and at eight weeks after surgery was 29.1 ng/dl (range: 13.2 to 50). Pathology report showed that almost 88% of the cases were caused by single or double adenomas (76% by single adenoma) and 12% by hyperplasic glands.

Conclusion: In our series, almost all our patients with PHPT had a single or a double adenoma, and not four gland hyperplasia. VDD improved without VD supplementation after cure of the PHPT. These results suggest that VDD does not cause PHPT due to the very low incidence of four gland hyperplasia.

Keywords

Hyperparathyroidism, Vitamin D deficiency, Parathyroid adenoma

Introduction

The overproduction of parathyroid hormone (PTH), termed hyperparathyroidism (HPT), can be categorized as primary, secondary, or tertiary [1]. Primary hyperparathyroidism (PHPT) arises from an unregulated overproduction of PTH from an abnormal parathyroid gland or glands [1]. The prevalence of PHPT has been rising since the advent of routine laboratory testing affecting one to seven people per 1000 persons per year (the prevalence has increased from 0.1% to 0.4%) in the United States of America [1-5]. It is the third most common endocrine diagnosis and the most common cause of hypercalcemia in the outpatient setting [1,6,7]. The vast majority of patients with PHPT are older than 45 years of age, with the mean age at diagnosis been between 52 and 59 years of age [1-3]. The risk of developing PHPT is five-times higher in women than in men with the female to male ratio of 3 to 4:1 [1,7]. The majority of cases (85% to greater than 90%) of PHPT are secondary to a single parathyroid adenoma [1,2]. The diagnosis of PHPT is usually made bi-

Citation: Arrangoiz R, Sánchez-García J, Moreno-Paquentin E, Molina DC, de León EL, et al. (2018) Does Vitamin D Deficiency Cause Primary Hyperparathyroidism?. Int J Surg Res Pract 5:064. doi.org/10.23937/2378-3397/1410064

Received: March 14, 2018: **Accepted:** March 29, 2018; **Published:** March 31, 2018

ochemically with the combination of hypercalcemia and an elevated or inappropriately normal PTH level [1,2,5]. Vitamin D deficiency (VDD) is a very common finding in patients with PHPT, with a reported incidence varying between 53% to 77% of the cases [1,2,5]. We defined vitamin D deficiency based on a paper by Norman, et al. [2], in which he defined the normal range above 35 ng/ml in series of more than 10,000 patients with proven PHPT. There is a misconception that patients with hypercalcemia, elevated PTH levels, and VDD that the origin of the elevated PTH level and the calcium level is secondary to the low levels of vitamin D. The aim of our study is to describe a cohort of patients with PHPT with or without VDD and to show that the vast majority of patients with PHPT (proven with biopsy of the abnormal parathyroid gland) have single gland disease with VDD and not four gland hyperplasia as it to be expected if the origin of the PHPT was the VDD.

Materials and Methods

This is a retrospective study from a prospectively kept database of patients with PHPT treated by our group from January 2015 to July 2017 at the American British Cowdray Medical Centre in Mexico City. The operation performed by our group consisted of a bilateral neck exploration through a two centimeter incision, and a radioguided parathyroidectomy as described previously by various authors [8-15]. We continue to use intraoperative PTH levels (baseline, 10, and 20 minutes after a resection) [11,16]. The patient demographics that were obtained from our electronic medical records included age, gender, preoperative, 24 hours, 8 weeks postoperative serum calcium and PTH levels. The preoperative, postoperative vitamin D levels and the pathology results were also reviewed. The data was analysed calculating the absolute and relative frequencies, measure of central tendency according to the variables. Patients without complete medical records were not included. All data were collected in a non-identifiable fashion in accordance with the principles outlined in the Declaration of Helsinki and as required for our institutional review board approval [17].

Results

A total of 50 patients with PHPT were included in our study. Four fifths of the patients were females and one fifth were males; the mean age at diagnosis for our study population was 56.7 years (range: 29 to 87). The mean preoperative PTH was 97 pg/ml (range: 26.5 to 247), 24 hours postoperative value was 38.7 pg/ml, and at eight weeks after surgery 55.2 pg/ml. The mean preoperative calcium level was 10.2 mg/dl (range: 8.8 to 11.9), at 24 hours and eight weeks after surgery it was 8.6 mg/dl and 9.4 mg/dl, respectively. Thirty-five patients (70%) had preoperatively VDD before surgery and had subjective exacerbation of their PHPT symptoms when correction was attempted preoperatively. The mean preoperative VD level was 27.5 ng/dl and at eight weeks after surgery it was 29.1 ng/dl (range: 13.2 to 50). The final pathology report showed that almost 88% of the cases were caused by a single or double adenoma (76% of the cases by a single adenoma) and 12% of the cases by four gland hyperplasia.

Discussion

PHPT is defined as hypercalcemia or widely fluctuating serum calcium levels resulting from the inappropriate or autogenous secretion of PTH by one of more parathyroid glands in the absence of known or recognized stimulus [1,2,18]. Approximately 100,000 new cases per year are reported in the United States [1,3,18]. The exact origin of PHPT is unknown, although exposure to low-dose therapeutic ionizing radiation [19,20] and familial predisposition account for some cases [21]. Various genetic aberrations have been identified in the development of PHPT, including anomalies in the tumor suppressor genes and proto-oncogenes. Certain DNA mutations in the parathyroid cell may confer a proliferative advantage over normal neighbouring cells, thus allowing for clonal growth [22]. Large populations of these altered cells containing the same mutation within hyper functioning parathyroid tissue suggest that such glands are a result of clonal expansion [1,22]. The majority of PHPT cases are sporadic, nevertheless, PHPT also occurs within the spectrum of a number of inherited disorders such as multiple endocrine neoplasia syndromes (MEN), MEN type 1 (Wermer Syndrome) [23], MEN type 2A (Sipple Syndrome) [24], isolated familial HPT [25,26], and familial HPT with jaw-tumor syndrome [26]. All of these are inherited in an autosomal dominant manner.

Regardless of what most of the literature reports, PHPT is symptomatic in more than 95% of the cases if proper attention is payed to the subtle symptoms and signs that this disease can produce due to the fluctuating calcium levels [1,27]. PHPT is diagnosed biochemically with the combination of hypercalcemia and an elevated or inappropriately normal PTH levels [1,2,18]. The majority of patients with PHPT will have concomitant VDD, with a reported incidence varying between 53% to 77% of the cases [1,2]. In our serious 70% of our patients (35 patients) had preoperatively VDD. This compares to a series published by Norman, et al. [2]. In these series of more than 10,000 patients with proven PHPT found that 77% of patients had 25 OH Vitamin D levels below 30 ng/ml (normal range above 35 ng/ml), 36% had levels below 20 ng/ml, and none of the patients had elevated 25 OH Vitamin D levels. VDD is something to expect in patients with PHPT with an average value of 22.4 ng/ml [2]. In our study 70% of our patients had preoperatively VDD before surgery with a mean preoperative VD level of 27.5 ng/dl. The patients in our cohort with VDD had subjective exacerbation of their PHPT symptoms when correction was attempted preoperatively. These patients had hypercalcemia and VDD, we usually do not replace VDD in patients with overt hypercalcemia.

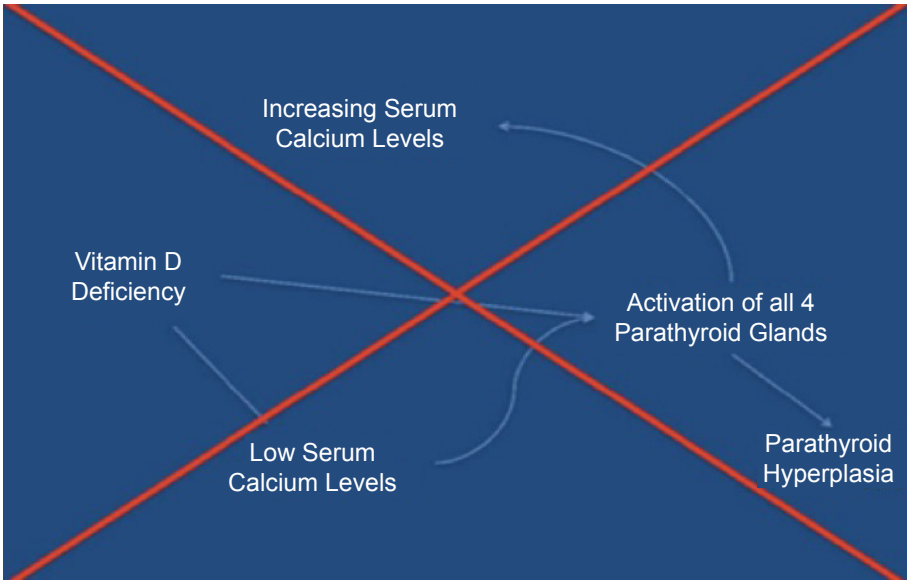

Figure 1: Erroneous pathophysiology of secondary hyperparathyroidism.

Fuller Albright in the 1930s and 1940s performed comprehensive amount of research on the physiology of the parathyroid glands, increasing our understanding of the complex interaction and homeostasis of calcium metabolism [28,29]. Nevertheless, it was not until the 1960s that importance of vitamin D in parathyroid disease was reported in a case with concomitant osteomalacia and PHPT [30]. VDD has been reported to affect roughly 14% of the healthy adult population during the winter period [31]. As mentioned previously VDD and PHPT are relatively common, in a study by Kantorovitch, et al. [32], comprising 229 patients referred for evaluation of low bone mineral density (BMD) revealed a prevalence of 2.2% for co-existing VDD and PHPT in their cohort. VDD seems to affect the severity of PHPT. Silverberg, et al. [33], assessed the correlation of vitamin D levels on disease severity in 124 patients with PHPT. Those with the lowest levels of vitamin D (25-hidroxy vitamin D/25 OHD) had higher PTH concentrations and greater bone turnover on biopsy studies. In another study of 148 patients with PHPT, Rao, et al. [34], showed an inverse correlation between serum 25-OHD and parathyroid gland weight. The presence of larger parathyroid adenomas in PHPT patients with VDD may explain the increase in disease severity [34].

Western countries in the 20th century had significant improvements in vitamin D supplementation, which lead to a change in the clinical presentation of PHPT [33]. Osteitis fibrosa cystica (seen in less than 5% of the cases of PHPT) became a rare manifestation of the disease and the levels of PTH and the weight of the parathyroid adenomas decreased dramatically [1,33]. The relationship of VDD and PHPT is evident on two separate levels. First, irrespective of the clinical severity of PHPT, the disease seems to be more severe in those with concomitant VDD. Second, VDD seems to be more common in patients with PHPT than in geographically matched populations [1,2,33,34]. The association between VDD and PHPT has clear implications. Concomitant VDD and PHPT may cause the serum calcium level to fall into the normal range (normocalcemic PHPT), which can lead to diagnostic difficulties [33].

There is a misconception that patients with hypercalcemia, elevated PTH levels, and VDD that the origin of the elevated PTH level and the calcium level is secondary to the low levels of vitamin D. Low vitamin D levels do NOT cause high levels of serum calcium [1,2]. The idea that VDD causes a decrease in the serum calcium levels (because of the decrease intestinal absorption) and that this decrease in serum calcium concentration will lead to an activation of the all four parathyroid glands, with subsequent four-gland parathyroid hyperplasia leading to an increase in PTH secretion promoting hypercalcaemia should be reconsidered (Figure 1). Norman, et al. [2], revealed that 98% of the patients in their study who had PHPT with concomitant VDD had a parathyroid adenoma and only two percent had parathyroid gland hyperplasia rebutting the current thinking shown in (Figure 1). This held true in our study population in which the final pathology report showed that almost 88% of the cases were caused by a single or double adenoma (76% of the cases by a single adenoma) and 12% of the cases by four gland hyperplasia.

With regard to management, preliminary data on vitamin D repletion in patients with mild PHPT suggest that, in some cases, correction of VDD may be accomplished without worsening the underlying hypercalcemia [32,35,36], but other available data suggest that vitamin D repletion may worsen the hypercalcemia, the hypercalciuria, and the symptoms of PHPT, in some rare cases causing a hypercalcemic crisis [8,37-39]. The patients in our cohort with PHPT and VDD had subjective exacerbation of their symptoms when correction was attempted preoperatively, but this observation can be biased because we did not perform formal questionnaires on symptoms before and after surgery.

Several studies have focused on the repletion of VDD in PHPT, and often with addition of patients with mild and minimally symptomatic or in the rare cases of truly asymptomatic disease not meeting international criteria for surgery. The bigger studies are typical prospective but uncontrolled or historical observational studies. In these studies, the change in clinical diagnosis, laboratory diagnosis, and management over time may affect the results leading to bias in their conclusions. Shah, et al. [40], performed a meta-analysis of the world literature in 2014 and concluded Vitamin D replacement in subjects with PHPT and coexistent VDD increased 25 OHD and reduces serum PTH significantly without causing hypercalcemia and hypercalciuria but mentioned that the finding of their study needs to be confirmed by larger randomized control trials. It has been reported in the literature that replacement of vitamin D in patients with PHPT may or may not decrease PTH levels and bone turnover and potentially increase bone mass in various compartments [32,38,39]. However, some patients experience increasing plasma levels of calcium, triggering either vitamin D withdrawal or surgery [8,38,39]. The third international workshop on management of mild PHPT recommend vitamin D repletion [41]. However as described above the scientific evidence for this recommendation is weak. Only prospective, randomized and blinded, placebo-controlled studies can yield the evidence for vitamin D repletion or treatment in PHPT.

As will all retrospective series our study has several limitations: The retrospective aspect may introduce selection bias and misclassification or information bias, the temporal relationship is frequently difficult to assess. Retrospective studies may need very large sample sizes for rare outcomes and our sample size is limited compared to other authors. We did not measure 1,25-dihydroxyvitamin D concentrations this would had helped us see how the VDD improves without the need for supplementation after parathyroidectomy. Our group does not correct the VDD if symptomatic PHPT is diagnosed. In the setting of normal or low normal serum calcium concentrations with minimal elevations of the PTH levels (greater than 65 to less than 100 pg/dl) and minimally symptomatic disease, in which the diagnosis of normocalcemic PHPT is entertained but cannot be differentiated from VDD induced elevations we do correct the VDD (our goal is a 25 OHD above 35 to 40 ng/dl), but with close surveillance of the patients to make a quick diagnosis of any exacerbating symptoms in a timely fashion and treat the normocalcemic PHPT appropriately with surgery.

Conclusion

In our series, most patients had PHPT secondary to adenomas, not four-gland hyperplasia. VDD improved without VD supplementation after resection of the adenomas. These results suggest that VDD does not cause the PHPT but is a common finding that can help with the diagnosis. Care must be taken when supplementation with vitamin D is recommended in patients with normal or low normal serum calcium concentrations with minimal elevations of the PTH levels in which the diagnosis of normocalcemic PHPT is entertained but cannot be differentiated from VDD.

Competing Interests

The author(s) declare that they have no competing interests.

The authors have no non-financial competing interests (political, personal, religious, ideological, academic, intellectual, commercial or any other) to declare in relation to this manuscript.

Authors Contributions

Idea: Rodrigo Arrangoiz

Literature review, data collection, initial manuscript elaboration, and editing: Rodrigo Arrangoiz, Luis Fernando Negrete, Jorge Sánchez-García

Final manuscript elaboration: Rodrigo Arrangoiz

Review and editing of manuscript: Rodrigo Arrangoiz, David Caba, Fernando Cordera, Luis Fernando Negrete, Eduardo Moreno, Enrique Luque, Jorge Sánchez-García, Efrain Cruz, Manuel Muñoz

The results of this manuscript were presented in an oral presentation at the Academic Surgical Congress conference held in Jacksonville, Florida in January 2018.

References

1. Arrangoiz R, Lambreton F, Luque-de-Leon E, Fernando Cordera, Muñoz-Juárez M, et al. (2016) Current Thinking on Primary Hyperparathyroidism. JSM Head Neck Cancer Cases Rev 1: 1002.
2. Norman J, Goodman A, Politz D (2011) Calcium, parathyroid hormone, and vitamin D in patients with primary hyperparathyroidism: normograms developed from 10,000 cases. Endocr Pract 17: 384-394.
3. Wermers RA, Khosla S, Atkinson EJ, Achenbach SJ, Oberg AL, et al. (2006) Incidence of primary hyperparathyroidism in Rochester, Minnesota, 1993-2001: an update on the changing epidemiology of the disease. J Bone Miner Res 21: 171-177.
4. Adami S, Marcocci C, Gatti D (2002) Epidemiology of primary hyperparathyroidism in Europe. J Bone Miner Res 17: 18-23.
5. Habib Z, Camacho P (2010) Primary hyperparathyroidism: an update. Curr Opin Endocrinol Diabetes Obes 17: 554-560.
6. Khan AA, Hanley DA, Rizzoli R, Bollerslev J, Young JE, et al. (2017) Primary hyperparathyroidism: review and recommendations on evaluation, diagnosis, and management. A Canadian and international consensus. Osteoporos Int 28: 1-19.
7. Palmieri S, Roggero L, Cairoli E, Morelli V, Scillitani A, et al. (2017) Occurrence of malignant neoplasia in patients with primary hyperparathyroidism. Eur J Intern Med 43: 77-82.

8. Norman J, Lopez J, Politz D (2012) Abandoning unilateral parathyroidectomy: why we reversed our position after 15,000 parathyroid operations. J Am Coll Surg 214: 260-259.

9. Norman J, Politz D (2010) Prospective study in 3,000 consecutive parathyroid operations demonstrates 18 objective factors that influence the decision for unilateral versus bilateral surgical approach. J Am Coll Surg 211: 244-249.

10. Norman J, Politz D (2009) 5,000 parathyroid operations without frozen section or PTH assays: measuring individual parathyroid gland hormone production in real time. Ann Surg Oncol 16: 656-666.

11. Beatriz de Rienzo-Madero (2017) The return of the bilateral neck exploration for primary hyperparathyroidism. Medical Science 21: 305-316.

12. Norman J, Chheda H, Farrell C (1998) Minimally invasive parathyroidectomy for primary hyperparathyroidism: decreasing operative time and potential complications while improving cosmetic results. Am Surg 64: 391-395.

13. Costello D, Norman J (1999) Minimally invasive radioguided parathyroidectomy. Surg Oncol Clin N Am 8: 555-564.

14. Murphy C, Norman J (1999) The 20% rule: a simple, instantaneous radioactivity measurement defines cure and allows elimination of frozen sections and hormone assays during parathyroidectomy. Surgery 126: 1023-1028.

15. Flynn MB, Bumpous JM, Schill K, McMasters KM (2000) Minimally invasive radioguided parathyroidectomy. J Am Coll Surg 191: 24-31.

16. Carter AB, Howanitz PJ (2003) Intraoperative testing for parathyroid hormone: a comprehensive review of the use of the assay and the relevant literature. Arch Pathol Lab Med 127: 1424-1442.

17. Helsinki (2004) Ethical principles for medical research involving human subjects.

18. Fraser WD (2009) Hyperparathyroidism. Lancet 374: 145-158.

19. Beard CM, Heath H, O'Fallon WM, Anderson JA, Earle JD, et al. (1989) Therapeutic radiation and hyperparathyroidism. A case-control study in Rochester, Minn. Arch Intern Med 149: 1887-1890.

20. Fujiwara S, Sposto R, Ezaki H, Akiba S, Neriishi K, et al. (1992) Hyperparathyroidism among atomic bomb survivors in Hiroshima. Radiat Res 130: 372-378.

21. Arnold A, Shattuck TM, Mallya SM, Krebs LJ, Costa J, et al. (2002) Molecular pathogenesis of primary hyperparathyroidism. J Bone Miner Res 17: 30-36.

22. Arnold A, Staunton CE, Kim HG, Gaz RD, Kronenberg HM (1988) Monoclonality and abnormal parathyroid hormone genes in parathyroid adenomas. N Engl J Med 318: 658-662.

23. Thakker RV, Newey PJ, Walls GV, Bilezikian J, Dralle H, et al. (2012) Clinical practice guidelines for multiple endocrine neoplasia type 1 (MEN1). J Clin Endocrinol Metab 97: 2990-3011.

24. Calmettes C, Ponder BA, Fischer JA, Raue F (1992) Early diagnosis of the multiple endocrine neoplasia type 2 syndrome: consensus statement. European Community Concerted Action: Medullary Thyroid Carcinoma. Eur J Clin Invest 22: 755-760.

25. Hannan FM, Nesbit MA, Christie PT, Fratter C, Dudley NE, et al. (2008) Familial isolated primary hyperparathyroidism caused by mutations of the MEN1 gene. Nat Clin Pract Endocrinol Metab 4: 53-58.

26. Simonds WF, Robbins CM, Agarwal SK, Hendy GN, Carpten JD, et al. (2004) Familial isolated hyperparathyroidism is rarely caused by germline mutation in HRPT2, the gene for the hyperparathyroidism-jaw tumor syndrome. J Clin Endocrinol Metab 89: 96-102.

27. Perrier ND (2005) Asymptomatic hyperparathyroidism: a medical misnomer? Surgery 137: 127-131.

28. Albright F, Walter Bauer, Jessie Reed Cockrill, Read Ellsworth (1931) Studies on the Physiology of the Parathyroid Glands: II. The Relation of the Serum Calcium to the Serum Phosphorus at Different Levels of Parathyroid Activity. J Clin Invest 9: 659-677.

29. Albright F, Walter Bauer, Jessie Reed Cockrill, Read Ellsworth (1932) Studies In Parathyroid Physiology: Iii. The Effect of Phosphate Ingestion in Clinical Hyperparathyroidism. J Clin Invest 11: 411-435.

30. Vaishava H, Rizvi SN (1969) Primary hyperparathyroidism associated with nutritional osteomalacia. Am J Med 46: 640-644.

31. Chapuy MC, Preziosi P, Maamer M, Arnaud S, Galan P, et al. (1997) Prevalence of vitamin D insufficiency in an adult normal population. Osteoporos Int 7: 439-443.

32. Kantorovich V, Gacad MA, Seeger LL, Adams JS (2000) Bone mineral density increases with vitamin D repletion in patients with coexistent vitamin D insufficiency and primary hyperparathyroidism. J Clin Endocrinol Metab 85: 3541-3543.

33. Silverberg SJ (2007) Vitamin D deficiency and primary hyperparathyroidism. J Bone Miner Res 2: 100-104.

34. Rao DS, Honasoge M, Divine GW, Phillips ER, Lee MW, et al. (2000) Effect of vitamin D nutrition on parathyroid adenoma weight: pathogenetic and clinical implications. J Clin Endocrinol Metab 85: 1054-1058.

35. Grey A, Lucas J, Horne A, Gamble G, Davidson JS, et al. (2005) Vitamin D repletion in patients with primary hyperparathyroidism and coexistent vitamin D insufficiency. J Clin Endocrinol Metab 90: 2122-2126.

36. Tucci JR (2009) Vitamin D therapy in patients with primary hyperparathyroidism and hypovitaminosis D. Eur J Endocrinol 161: 189-193.

37. Silverberg SJ, Shane E, Dempster DW, Bilezikian JP (1999) The effects of vitamin D insufficiency in patients with primary hyperparathyroidism. Am J Med 107: 561-567.

38. Grubbs EG, Rafeeq S, Jimenez C, Feng L, Lee JE, et al. (2008) Preoperative vitamin D replacement therapy in primary hyperparathyroidism: safe and beneficial? Surgery 144: 852-858.

39. Isidro ML, B Ruano (2009) Biochemical effects of calcifediol supplementation in mild, asymptomatic, hyperparathyroidism with concomitant vitamin D deficiency. Endocrine 36: 305-310.

40. Shah VN, Shah CS, Bhadada SK, Rao DS (2014) Effect of 25 (OH) D replacements in patients with primary hyperparathyroidism (PHPT) and coexistent vitamin D deficiency on serum 25(OH) D, calcium and PTH levels: a meta-analysis and review of literature. Clin Endocrinol (Oxf) 80: 797-803.

41. Bilezikian JP, Khan AA, Potts JT Jr (2009) Guidelines for the management of asymptomatic primary hyperparathyroidism: summary statement from the third international workshop. J Clin Endocrinol Metab 94: 335-339.