Normocalcemic primary hyperparathyroidism: a survey in a small village of Southern Italy

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Abstract

We investigated the prevalence of normocalcemic primary hyperparathyroidism (NPHPT) in the adult population living in a village in Southern Italy. All residents in 2010 ($n = 2045$) were invited by calls and 1046 individuals accepted to participate. Medical history, calcium intake, calcium, albumin, creatinine, parathyroid hormone (PTH) and 25OHD were evaluated. NPHPT was defined by normal albumin-adjusted serum calcium, elevated plasma PTH, and exclusion of common causes of secondary hyperparathyroidism (SHPT) (serum 25OHD < 30 ng/ml, estimated glomerular filtration rate (eGFR) < 60 ml/min per 1.73 m^2 and thiazide diuretics use), overt gastrointestinal and metabolic bone diseases. Complete data were available for 685 of 1046 subjects. Twenty subjects did not meet the inclusion criteria and 341 could not be evaluated because of thawing of plasma samples. Classical PHPT was diagnosed in four women (0.58%). For diagnosing NPHPT the upper normal limit of PTH was established in the sample of the population ($n = 100$) who had 25OHD $\geq$ 30 ng/ml and eGFR $\geq$ 60 ml/min per 1.73 m^2 and was set at the mean $+$ 3s.d. Three males (0.44%) met the diagnostic criteria of NPHPT. These subjects were younger and with lower BMI than those with classical PHPT. Our data suggest, in line with previous studies, that NPHPT might be a distinct clinical entity, being either an early phenotype of asymptomatic PHPT or a distinct variant of it. However, we cannot exclude that NPHPT might also represent an early phase of non-classical SHPT, since other variables, in addition to those currently taken into account for the diagnosis of NPHPT, might cumulate in a normocalcemic subject to increase PTH secretion.

Introduction

During the past 10–20 years several investigators have noticed that there are patients with elevated serum parathyroid hormone (PTH) levels associated with consistently normal serum calcium concentration and no other causes of secondary hyperparathyroidism (SHPT), and they described this entity as normocalcemic primary hyperparathyroidism (NPHPT) (1, 2, 3). This entity can only be recognized if PTH is measured in normocalcemic individuals. Vitamin D deficiency/insufficiency, the most common cause of SHPT, should be first to be excluded in

the diagnostic workout of NPHPT. Other causes of SHPT, such renal failure, hypercalciuria, gastrointestinal diseases associated with malabsorption and other metabolic bone diseases that could affect PTH levels (e.g. Paget's disease) should be excluded. Finally, the use of medications which might affect PTH levels or calcium metabolism (estrogens, thiazide diuretics, lithium, bisphosphonates, denosumab and anticonvulsants) should also be ruled out (4).

Few data are available on the prevalence of NPHPT, and most information has been obtained from subjects undergoing evaluation for skeletal health or selected populations (5, 6, 7).

The aim of the present study was to investigate the prevalence of NPHPT in the adult population living in a small village in Southern Italy.

Subjects and methods

The survey was conducted in the late summer of 2010 in Pescopagano, a village in Southern Italy, together with a study on the prevalence of thyroid disorders (8). All residents registered in 2010 ($n=2045$) were invited by two consecutive calls and 1046 adults participated in the study (Fig. 1). Informed consent was obtained from all participants.

All subjects underwent a medical interview, focused on their medical history, the use of calcium, vitamin D supplementation and anti-osteoporotic dugs. Daily calcium intake was estimated using a self-administered questionnaire (9).

Quantitative ultrasound scanning (QUS) at calcaneus was performed using a Hologic Sahara device (Hologic, Bedford, MA, USA). Speed-of-sound (SOS, in m/s) and broadband ultrasound attenuation (BUA, in dB/MHz) and stiffness index were calculated.

Blood samples were obtained by venipuncture and serum and plasma aliquots were stored at $-20\,^\circ$C.

Serum calcium, albumin and creatinine were measured using standard methods. Albumin-adjusted total serum calcium (alb-sCa) was calculated (10); the normal range was 8.6–10.2 mg/dl. Plasma PTH was measured by the Liason N-Tact PTH II (DiaSorin, Inc., Sitllwater, MN, USA); the intra-assay and inter-assay coefficient of variation (CV) at 10 pg/ml (lower normal value of the kit) and 65 pg/ml (upper normal value) were 7.2 and 4.0%, and 10.8 and 6.2%, respectively. Serum 25OHD was measured by RIA (DiaSorin, Inc.); the intra-assay and inter-assay CV at 10 and 30 ng/ml were 8.1 and 10.1%, and 7.8 and 9.0%, respectively. Vitamin D sufficiency was defined by serum 25OHD levels

≥ 30 ng/ml, as suggested by the Endocrine Society (11) and several opinion leaders (12, 13). Previous studies have shown that plasma PTH starts to raise when serum 25OHD concentration is below this value (14, 15). The estimated glomerular filtration rate (eGFR) was evaluated from serum creatinine (16).

The diagnosis of PHPT was based on increased alb-sCa, associated with elevated or inappropriately normal intact PTH. The diagnosis of NPHPT was based on the finding of normal alb-sCa and abnormally increased plasma PTH levels – the upper normal limit was calculated as detailed below – provided that serum 25OHD concentration was ≥ 30 ng/ml and the eGFR ≥ 60 ml/min per 1.73 m^2 and that subjects did not have overt gastrointestinal and metabolic bone diseases nor were taking thiazide diuretics.

Statistical analysis

All comparative analyses were performed with an intention-to-treat approach. Normality was assessed using the Shapiro–Wilk test, histograms and Q–Q plots. Continuous variables were expressed as means and s.D. Between-group differences were evaluated using the independent-samples t-test or ANOVA for continuous variables and the χ^2 test or Fisher's exact test for categorical variables, as appropriate. All P values were two-tailed and the minimum level of statistical significance was set at $P<0.05$. Statistical analyses were performed with the use of SAS software, version 8.2 (SAS Institute, Cary, NC, USA).

Results

The study group consisted of 1046 adult subjects (642 women, 404 men; mean age 48.2 ± 17.4, median 48.0, range 18–89 years) (Fig. 1). Complete medical and biochemical data were available in 685 subjects (66.8%); ultrasound studies were missing in 46 of them. Herein we refer to this sample of subjects as 'fully evaluated' (Fig. 1).

This cohort included 419 women and 266 men. The age did not differ between women and men. This cohort was comparable in terms of age and gender with the 1026 fully evaluated subjects. The complete biochemical and QUS evaluation is reported in Table 1.

Four women (0.58%) had classic hypercalcemic PHPT and 581 had SHPT (Fig. 1, Table 2).

We focused the following studies on the remaining 100 subjects, who had normal alb-sCa, serum 25OHD ≥ 30 ng/ml and/or eGFR ≥ 60 ml/min per 1.73 m^2, in order to identify those with abnormally elevated plasma PTH, namely the subjects who will meet the diagnostic

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          ┌─────────────────────┐
          │  Invited (n=2045)   │
          └──────────┬──────────┘
                     │      ┌──────────────────────────────┐
                     ├─────▶│  Did not respond (n=897)     │
                     │      └──────────────────────────────┘
          ┌──────────▼──────────┐
          │    Accepted to      │
          │ participate (n=1148)│
          └──────────┬──────────┘
                     │      ┌──────────────────────────────┐
                     ├─────▶│  Not evaluated: 102 subjects  │
                     │      │       aged <18 years          │
                     │      └──────────────────────────────┘
          ┌──────────▼──────────┐
          │ Evaluated (n=1046)  │
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Excluded by medical history (n=20)
• Bisphosphonates (n=13)
• Thiazide diuretics (n=2)
• Pregnancy (n=2)
• Chronic renal failure (n=3)

Fully investigated (n=1026)
• Daily calcium intake questionnaire
• Blood sampling
• Calcaneus quantitative ultrasound scanning

Not further evaluated because of thawing of plasma samples for PTH assay (n=341)

Fully evaluated (n=685)

Primary hyperparathyroidism (n=4)

Secondary hyperparathyroidism (n=581)
• 25OHD <30 ng/ml and eGFR <60 ml/min per 1.73 m^2 (n=43)
• 25OHD <30 ng/ml (n=531)
• eGFR <60 ml/min per 1.73 m^2 (n=7)

Normal 25OHD (≥30 ng/ml) and eGFR ≥60 ml/min per 1.73 m^2 (n=100)

Subjects with normal calcium and PTH (n=97)
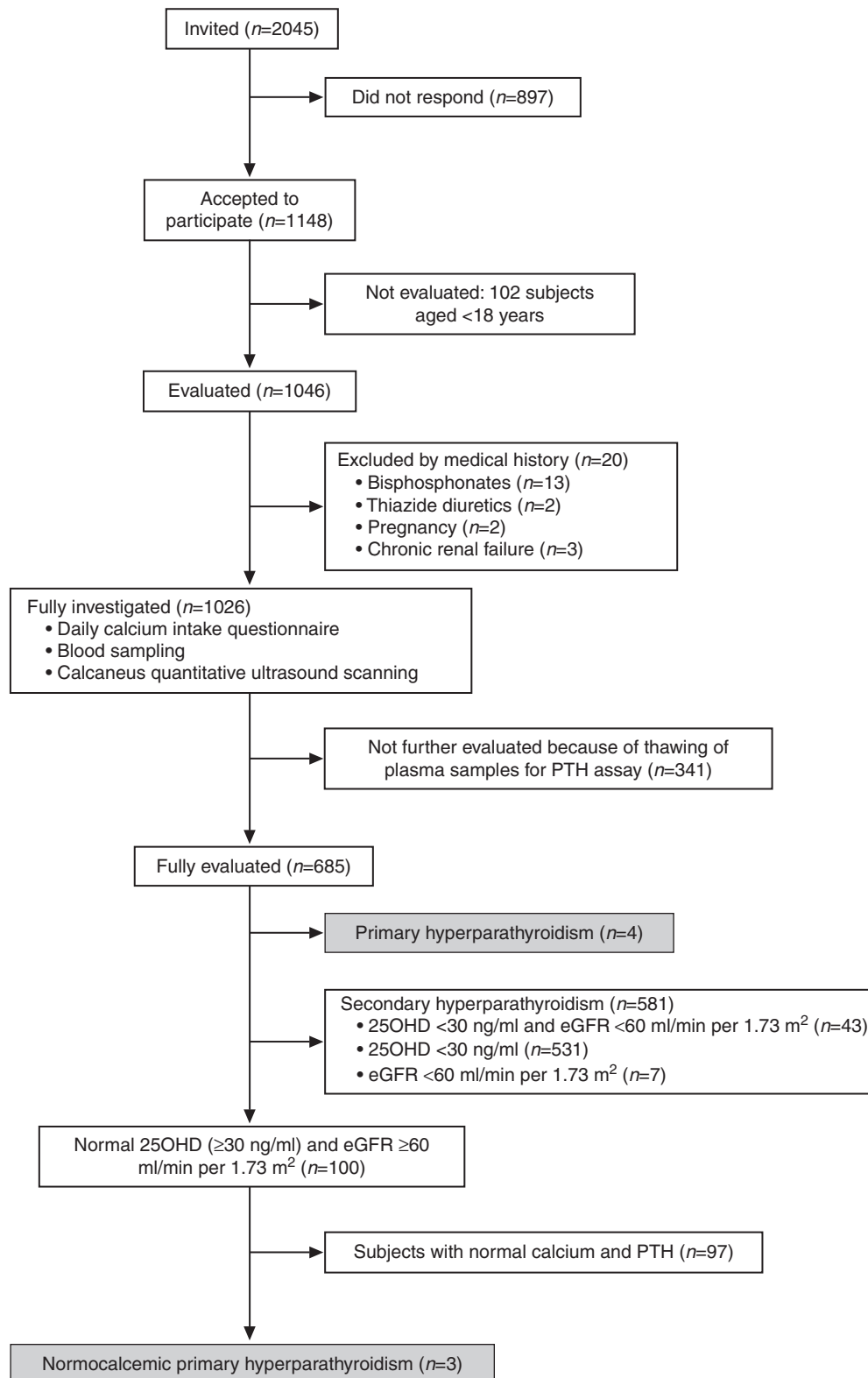
Normocalcemic primary hyperparathyroidism (n=3)

Figure 1

Diagram of the study population and evaluation. Plasma PTH levels were normally distributed in these 100 subjects; the upper limit of the normal range of plasma PTH was set at the mean + 3s.d.

Table 1 Characteristics of fully evaluated subjects and subgroups of subjects with normal 25OHD ($\geq$30 ng/ml) and normal eGFR ($\geq$60 ml/min per 1.73 m^2) and secondary hyperparathyroidism.

Parameter	Fully evaluated (*n*=685)	Secondary hyperparathyroidism (*n*=581)[a]	Normal 25OHD and eGFR (*n*=100)
Age (years)	47.6$\pm$17.4	48.2$\pm$17.3	43.2$\pm$17.6
Sex			
Female pre-MP	262	221	40
Female post-MP	157	138	16
Male	266	222	44
BMI (kg/m^2)	27.8$\pm$5.2	28.1$\pm$5.3	26.2$\pm$4.3
Estimated daily calcium intake (mg)	754$\pm$472	755$\pm$486	763$\pm$403
eGFR (ml/min per 1.73 m^2)	94.6$\pm$30.0	94.9$\pm$30.6	92.3$\pm$22.5
Alb-sCa (mg/dl)	9.1$\pm$0.5	9.0$\pm$0.5	9.1$\pm$0.3
Plasma PTH (pg/ml)	68.2$\pm$35.1	68.9$\pm$33.9	58.9$\pm$23.1
Serum 25OHD (ng/ml)	20.3$\pm$10.5	17.4$\pm$7.6	37.9$\pm$7.7
Calcaneal US[b]			
SOS (m/s)	1527$\pm$41	1526$\pm$41	1533$\pm$35
BUA (dB/MHz)	67.3$\pm$20.4	67.0$\pm$20.9	69.3$\pm$17.0
Stiffness index	82.5$\pm$24.4	82.1$\pm$24.8	84.9$\pm$21.7

Pre-MP, premenopausal; post-MP, postmenopausal; eGFR, estimated glomerular filtration rate; alb-sCa, albumin-adjusted serum calcium; SOS, speed of sound; BUA, broadband ultrasound attenuation.
[a]Four patients had primary hyperparathyroidism.
[b]Data were available in 639 subjects.

criteria of NPHPT. In this context, the definition of the normal PTH range is of paramount importance. Plasma PTH levels were normally distributed in these 100 subjects, and we decided to set the upper normal limit at the mean+3s.d. Contrary to what could be expected when values are normally distributed, three subjects, all males, had plasma PTH levels above this cut-off value (and therefore met the diagnostic criteria of NPHPT. Thus, the prevalence rate of NPHPT was three out of 685 (0.44%).

The clinical and biochemical parameters of the three subjects with NPNPT are reported in Table 2 together with those of the four patients with classical PHPT and normal controls.

Discussion

NPHPT is a new clinical entity identified by the widespread use of PTH assay in the evaluation of skeletal health,

Table 2 Characteristics of subjects with normocalcemic primary hyperparathyroidism, hypercalcemic primary hyperparathyroidism and controls[a].

ID	Sex	Age (years)	BMI (kg/m^2)	Estimated daily calcium intake (mg)	eGFR (ml/min per 1.73 m^2)	alb-sCa (mg/dl)	Plasma PTH (pg/ml)	Serum 25OHD (ng/ml)
Normocalcemic primary hyperparathyroidism (*n*=3)								
FM	M	21	21.0	669	90.9	8.9	130	37.4
MM	M	56	27.3	107	73.7	8.8	131	32.2
FS	M	64	28.3	1205	125	8.9	139	42.8
Mean$\pm$s.d.		47.0$\pm$22.9	25.5$\pm$3.9	660$\pm$549	96.5$\pm$26.1	8.9$\pm$0.1	133$\pm$5	37.5$\pm$5.3
Hypercalcemic primary hyperparathyroidism (*n*=4)								
MA	F	86	25.8	428	124.5	10.6	119	19.4
GL	F	52	24.0	160	109.6	10.5	130	21.2
MP	F	68	36.2	508	68.0	10.8	167	6.5
AC	F	59	30.0	857	79.3	11.1	382	14.8
Mean$\pm$s.d.		66.2$\pm$14.7	29.0$\pm$5.4	488$\pm$287	95.3$\pm$26.2	10.7$\pm$0.2	199$\pm$123	15.5$\pm$6.6
Controls (*n*=97)								
Mean$\pm$s.d.		43.1$\pm$17.1	26.2$\pm$4.4	748$\pm$395	90$\pm$21.3	9.1$\pm$0.3	58.9$\pm$23.1	37.9$\pm$7.7

eGFR, estimated glomerular filtration rate; alb-sCa, albumin-adjusted serum calcium.
[a]Controls are subjects with normal alb-sCa and PTH, 25OHD$\geq$30 ng/ml and eGFR $\geq$60 ml/min per 1.73 m^2.

particularly in postmenopausal women (4). This condition, characterized by elevated PTH levels and normal total and ionized serum calcium, and no other causes of SHPT, can only be recognized by measuring PTH in normocalcemic individuals (4).

The epidemiology of NPHPT has been investigated in various populations (5, 6, 7). Most studies were performed in selected gender and age groups, and different diagnostic criteria were used. Moreover, not all causes of SHPT were excluded. Finally, with the exception of the study of Lundgren *et al.* (7), ionized calcium was not measured. Therefore, the available data might overestimate the true prevalence of NPHPT.

In the present study the evaluation of the prevalence of NPHPT in an unselected sample of the whole community of adults living in a small village in Southern Italy (*n*=685 (37.2%)) could give a more accurate estimate of the true prevalence of NPHPT. The key points in addressing this issue are to exclude all secondary causes of elevated PTH levels and to define the upper limit of normal plasma PTH. The upper normal limit of PTH is usually fixed at the 97.5th percentile; therefore, some individuals with slightly elevated PTH might be wrongly classified as NPHPT. The novelty of our study is that we defined the upper normal limit of PTH in the same population in which we performed the study and that it was set at the mean+3s.d. (99th percentile). The choice of the mean+3s.d. for defining the upper normal PTH level makes it unlikely that we classified as NPHPT normal subjects. According to this criterion, the estimated prevalence of NPHPT in our series was three out of 685 (0.44%) subjects. If we adopted the most commonly approach of 2s.d. (97.5th percentile) for defining the upper normal limit of PTH five subject instead of three would have been classified as NPHPT, leading to a prevalence of 0.73%.

The prevalence of NPHPT in our study is lower than that reported by Cusano *et al.* (6) (3.1%) in an unselected population-based cohort (Dallas Hearth Study), despite the use of a lower cut-off value of 25OHD (≥20 ng/ml). In another population-based study (Canadian Multicenter Osteoporosis Study) Berger *et al.* found a greater prevalence (16.7%), using the same cut-off value of serum 25OHD (≥20 ng/ml), but not excluding other causes of SHPT (17). However, if in the latter population the analysis is limited to subjects whose 25OHD was >30 ng/ml, the prevalence of NPHPT falls to 6.9%, a figure closer to that found by Cusano *et al.* (6) but definitely higher that that found in our study. Other studies in selected populations have shown a prevalence of

NPHPT ranging between 0.4 and 6% (5, 6, 7, 18). Differences in age, gender, ethnicity and criteria to exclude SHPT may account for these findings.

All patients with NPHPT were males. A similar but not significantly different gender distribution was reported by Cusano *et al.* (6). Conversely, no gender difference was observed by Berger *et al.* (19).

A regular monitoring of subjects with NPHPT is advisable. Indeed, some may remain stable for years with persistently elevated plasma PTH levels and normal serum calcium, whereas others may progress to a classical form PHPT, with development of hypercalcemia and target organ involvement (6, 20, 21). Conversely, as shown by Cusano *et al.*, a significant proportion of subjects with provisional diagnosis of NPHPT would no longer fit this diagnosis during follow-up. As a matter of fact, upon retesting after approximately 8 years, 64 of the 108 subjects initially classified as NPHPT, only 13 (20%) continued to show biochemical findings of NPHPT, 20 no longer met the NPHPT criteria because of other causes of SHPT, one developed hypercalcemic PHPT, one showed hypercalcemia in the setting of renal failure, and 29 had normal PTH (6). Therefore, the prevalence of NPHPT fell from 3.1 to 0.6% of the total cohort with follow-up data. The latter finding underscores the need for biochemical monitoring of patients with a provisional diagnosis of NPHPT in order to confirm the diagnosis.

We would like to add a word of caution when evaluating a subject with a suspected NPHPT based upon established criteria (1, 6): other variables which may increase PTH secretion (which are not currently taken into account for the diagnosis of NPHPT), such as age, BMI, waist circumference, calcium intake, subclinical gastrointestinal disorders, etc. might cumulate in an individual patient to trigger hyperparathyroidism. Therefore, the possibility that NPHPT may represent an early phase of non-classical SHPT cannot be excluded. Thus, in borderline cases retesting after vitamin D and calcium supplementation may be advisable. Finally, the use of an oral peptone and calcium load test might be useful in selected cases to differentiate patients with NPHPT from those with PHPT (22).

Our study has some limitations: i) the entire adult village population was not investigated; ii) complete information regarding the current medical therapy was unavailable in few patients; iii) ionized serum calcium and urinary calcium excretion were not measured; iv) a single blood sample was drawn from each participating subject

and assays were performed in singlicate; v) no follow-up data were available.

We were unable to measure ionized serum calcium and 24-h urinary calcium excretion, which are important parameters for a secure diagnosis of NPHPT. As far as ionized serum calcium is concerned, we recognize that, although there is in general a linear relationship between albumin-corrected serum calcium and ionized calcium, some discrepancies may occur (23). However we believe that this should not be the case in our three subjects classified as NPHPT, because their albumin-corrected serum calcium was well below the upper normal limit, and this never occur in our clinical practice. We did not measure 24-h urinary calcium because we thought that it would have been very difficult to collect reliable 24-h urine sample in a population study like the present one. However, it is of note that all previous studies on the prevalence of NPHPT in the various populations so far reported did not include the measurement of this parameter. Finally, missing the diagnosis of familial hypocalciuric hypercalcemia (FHH) in our population would be insignificant due to the very low prevalence of FHH (1:10 000–1:100 000) (24).

Our study has the strength that we evaluated a population-based adult cohort, which included both females and males, and collected data on calcaneus ultrasound scanning. To our knowledge only two other studies have evaluated similar cohorts and in only one study was bone mineral density measured. Moreover, the normal range of plasma PTH was established in the same population studied, and a more conservative criterion of abnormally increased plasma PTH value (corresponding to the 99.7 percentile) was used.

In conclusion, despite the limitations mentioned above, our data provide further support for the recognition of NPHPT as a new clinical entity, which may be either an early phenotype of asymptomatic PHPT or a distinct variant of it. However, we cannot exclude that the biochemical findings suggestive of NPHPT might also represent an early phase of non-classical SHPT, since other variables, in addition to those currently taken into account for the diagnosis of NPHPT, might cumulate in a normocalcemic subject to increase PTH secretion. Long-term follow-up studies of subjects with biochemical finding of NPHPT are needed to shed light on these issues.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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