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**ARAŞTIRMA / RESEARCH**

# *CDKN1B* mutation analyses and biochemical characteristics in patients with symptomatic or asymptomatic primary hyperparathyroidism

## Semptomatik veya asemptomatik primer hiperparatiroidisi olan hastaların biyokimyasal parametreleri ile *CDKN1B* mutasyon analizi tayini

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**Abstract**

**Purpose:** The aim of this study was to compare clinical, biochemical and treatment modalities of the patients with symptomatic and asymptomatic PHPT (primary hyperparathyroidism), and evaluate whether the *CDKN1B* mutation from these patients contributes to the pathogenesis of typical, sporadic parathyroid adenomas.

**Materials and Methods:** In this prospective study 80 patients (66 women and 14 men, mean age 50.8 ± 12.01 years) with PHPT were enrolled. Biochemical and clinical information were collected on patients' sex, age, biochemical examination and radiological findings (nuclear $^{99\,m}$Tc sestamibi scans scintigraphy, cervical ultrasound). *CDKN1B* sequencing, and DNA isolation was performed by using GeneMATRIX Quick Blood DNA Purification Kit. Selected primer of CDKN1BF (rs786201010, c.-456_-453delCCTT) (CAGGTTTGTTGGCAGCAGTA) and CDKN1BR (rs786201010, c.-456_-453delCCTT) (GGAGCCAAAAGACACAGACC) were amplified by polymerase chain reaction (PCR) (Solis Biodyne, Estonia).

**Results:** A total of 80 patients diagnosed with PHPT were included, of which 22 were symptomatic. Serum calcium and 24-hour calcium excretion were significantly increased in patients with symptomatic PHTP. Serum PTH levels were similar between the two group. PHPT. CDKN1B mutation was not detected in any patients.

**Conclusion:** Symptomatic patients were found to have elevated levels of calcium levels (hypercalcaemic), 24-hour urine calcium excretion and target organ damage (bone disease and nephrolithiasis). Independent of PTH levels, clinical signs and symptoms could be related with serum calcium parameters in these patients.

**Keywords:**. Primary Hyperparathyroidism, symptomatic hyperparathyroidism, asymptomatic hyperparathyroidism

**Öz**

**Amaç:** Bu çalışmada semptomatik ve asemptomatik primer hiperparatiroidi (PHPT) olgularını karşılaştırmayı amaçladık, beraberinde sporadik saptanan paratiroid adenomlarında etyopatogenezde CDKN1B mutasyonu varlılığını saptamaya çalıştık.

**Gereç ve Yöntem:** Çalışmamıza kliniğimize başvuran 80 PHPT (66 K ve 14 E, ortalama yaş 50.8 ± 12.01 yıl) tanısı almış hasta dahil edilmiştir. Hastaların yaş, cinsiyet, biyokimyasal parametreleri, görüntüleme yöntemleri (nükleer sintigrafi, ultrasonografi, kemik dansitometre ölçümü) kayıt edilmiştir. CDKN1B gen sekanslaması için GeneMATRIX Quick Blood DNA Purification kiti kullanılarak DNA izole edilmiştir. CDKN1BF (rs786201010, c.-456_-453delCCTT) (CAGGTTTGTTGGCAGCAGTA) ve CDKN1BR (rs786201010, c.-456_-453delCCTT) (GGAGCCAAAAGACACAGACC) primerleri seçilerek mutasyon analizi yapılmıştır.

**Bulgular:** Çalışma sonucunda 22 hasta asemptomatik PHPT olarak tanımlanmış olup semptomatik PHPT (n=68) serum kalsiyum parametreleri ve 24 saatlik idrar $Ca^{+}$ atılımı daha yüksek olarak saptanmıştır. Serum Parathormon (PTH) değerleri her iki grupta da benzerdi. Her iki grupta da CDKN1B mutasyonu açısından patolojik bir bulgu saptanmamıştır.

**Sonuç:** Parathormon seviyeleri semptomatik veya asemptomatik PHPT olgularında belirleyici bir parametre olmamakla birlikte semptomatik PHPT da serum kalsiyum değerleri ve 24 saatlik idrar $Ca^{+}$ atılımı daha belirgindir.

**Anahtar kelimeler**: Primer Hiperparatiroidi, semptomatik hiperparatiroidi, asemptomatik hiperparatiroidi

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## INTRODUCTION

The primary hyperparathyroidism (PHPT) is a common endocrine disease characterised by an excessive secretion of parathyroid hormone, and the elevated level of serum calcium is the determining factor of PHPT[1]. The clinical phenotype of PHPT changes from asymptomatic hypercalcemia to severe clinical form including overt bone and renal involvement[2]. PHPT is caused by solitary parathyroid adenoma in 80% of cases, whereas four-gland hyperplasia accounts for 10%–15%, multiple adenomas 5% and parathyroid carcinoma < 1% cases[3,4].

Patients with PHPT were generally discovered randomly in the context of biochemical screening and not for any signs or symptoms that would have prompted the health care provider to measure the serum calcium[5]. The surgical management of patients with PHPT has undergone considerable advances over the past two decades. However, several areas of controversy still remain including the indications for surgery, the extent of preoperative evaluation, the role of imaging studies or operative options [6,7]. There is a universal agreement that all symptomatic patients (renal or bone manifestations) should undergo surgical therapy[8,9,10]. Asymptomatic hyperparathyroidism, another variant of PHPT, has been the dominant clinical phenotype of PHPT for the past 40 years[11]. However, there is no information on the naturel history of asymptomatic PHPT about when or in whom surgical therapy is appropriate.

The genetic of PHPT is usually monoclonal when a single gland is involved and polyclonal when multiglandular disease is present[12]. Inherited or familial forms account for about 5% of cases that include multiple endocrine neoplasia types 1, 2A and 4, hyperparathyroidism–jaw tumour syndrome, familial isolated PHPT and familial hypocalciuric hypercalcaemia (FHH) [13-17]. Recent studies have also implicated *CDC73*, *CTNNB1*, *CDKN1B* and AIP (which encodes the aryl hydrocarbon receptor–interacting protein) in a small percentage of adenomas [18,19]. However, *CDKN1B* and other *CDKI* genes appear to be as genetic drivers of parathyroid tumorigenesis and may more commonly be involved in predisposition to sporadically presenting parathyroid tumours [20,21]. Most cases of symptomatic or asymptomatic PHPT have been still unclear regarding with the genetic mutations. Genetic and/or epigenetic mechanisms may explain the occurrence of symptomatic or asymptomatic PHPT.

Primary hyperparathyroidism may increase a patient's morbidity and even mortality if left untreated. During the last few decades, disease presentation has shifted from the classic presentation of severe bone and kidney manifestations to most patients now being diagnosed on routine labs. But there have been limited studies including symptomatic or asymptomatic PHPT.

We aim to compare clinical, biochemical and treatment modalities of the patients with symptomatic and asymptomatic PHPT. And we also evaluate whether the *CDKN1B* mutation from these patients contributes to the pathogenesis of typical, sporadic parathyroid adenomas. We also whether *CDKN1B* mutation from these patients contributes to the pathogenesis of typical, sporadic parathyroid adenomas were also analysed.

## MATERIALS AND METHODS

### Patients and procedure

In this prospective study 80 patients (66 women and 14 men, mean age 50.8 ± 12.01 years) with PHPT were enrolled between 2019 and 2020. This study was approved by the Cukurova University ethical committee in 2019 (No:92). The exclusion criteria were as follows: low vitamin D levels, other metabolic disease, known cancer, clinical or laboratory findings suggesting secondary or tertiary hyperparathyroidism, use of thiazide diuretics and lithium or active inflammatory granulomatous diseases.

All patients were evaluated serum calcium, phosphate, parathormon levels and 24 hour-urine Calcium levels. Clinical and biochemical information were collected on patients' sex, age, biochemical examination. If the results of biochemical parameters were correlated primary hyperparathyroidism, patients were evaluated for the radiological findings (nuclear $^{99m}$Tc sestamibi scans scintigraphy, cervical ultrasound).

We divided in to two categories in all patients with PHPT including symptomatic and asymptomatic.

Symptomatic PHPT was characterised by the following: evidence of hypercalcaemia (calcium reference range: 8.9–10.3 mg/dL), normal serum 25-

hydroxyvitamin D [25(OH) D > 20 ng/mL], elevated parathyroid hormone (PTH), organ-specific symptoms including renal disease, osteoporosis, mental symptoms, neuromuscular symptoms, gastrointestinal symptoms and cardiovascular diseases (22). Asymptomatic PHPT was characterised by elevated PTH levels without any target organ manifestations and clinical symptoms[23].

Patients with symptomatic and asymptomatic PHPT who met any guideline of the criteria were treated surgically [24]. Surgical therapy indications were as follows: age < 50 years, serum calcium > 1 mg/dL or 0.25 mmol/L of the upper limit of reference interval for total calcium and 0.12 mmol/L for ion calcium, impaired kidney function (glomerular filtration rate [GFR] < 60 mL/min, urinary calcium excretion > 400 mg/day), if stone or nephrocalcinosis, surgery should be recommended, osteoporosis (bone mineral density [BMD] T score< −2.5 standard deviation [SD]) at the lumbar spine, femoral neck and total hip.

## Imaging

Parathyroid imaging studies including $^{99}$ $^{m}$Tc sestamibi scans scintigraphy and ultrasound were performed in all patients. If parathyroid glands were not located by $^{99}$ $^{m}$Tc sestamibi scans scintigraphy, a combined technique ($^{99}$ $^{m}$Tc sestamibi scans scintigraphy and ultrasound) was performed to the patients. The presence of nephrolithiasis and bone fractures were screened. The results BMD and T score at the lumbar spine and left hip using dual-energy X-ray absorptiometry were also evaluated.

## Laboratory analysis

Blood calcium, phosphate, 24-hour urine calcium, albumin, creatinine by automated techniques was measured. The normal laboratory range was 8.9–10.3 mg/dL for calcium and 2.4–4.7 mg/dL for serum phosphate. Serum iPTH was measured by DXI Beckman Coulter Chemiluminescent Random Access Analyzer (Brea, CA, USA) following the manufacturer's protocol. The reference range for PTH level was 12–88 pg/mL. Serum 25-OHD was measured by radioimmunoassay (Thermo Scientific Ultimate 3000 UHPLC system).

## *CDKN1B* sequencing

Blood samples were obtained from 80 patients with PHPT, and DNA isolation was performed by using GeneMATRIX Quick Blood DNA Purification Kit (Poland). See detailed information in this link (http://eurx.com.pl/docs/manuals/en/e3565.pdf), and genomic DNA was checked by spectrophotometry (Thermo Scientific Nanodrop 2000 Coulter). Selected primer of CDKN1BF (rs786201010, c.-456_-453delCCTT) (CAGGTTTGTTGGCAGCAGTA) and CDKN1BR (rs786201010, c.-456_-453delCCTT) (GGAGCCAAAAGACACAGACC) were amplified by polymerase chain reaction (PCR) (Solis Biodyne, Estonia). The amplifications were performed in 40 cycles, each cycle consisting of denaturation at 95˚C for 45 seconds, annealing at 57˚C for 45 seconds and extension at 72°C for 60 seconds. PCR was carried out in reaction volumes of 35 μL, containing 2 μL of genomic DNA template, 0.3 μM each primer, 200 μM dNTP mix, 1.5 mM $MgCl_2$ and 2 units of Taq polymerase. PCR mixtures were run on 1.5% polyacrylamide gels in 1 × TAE for 90 minutes after amplification. PCR products were purified and subject to direct sequencing using a MAGBIO HighPrep PCR Clean-up System (AC-60005) Cycle Sequencing Kit (Applied Biosystems, Foster City, CA, USA) from an ABI 3730XL Genetic Analyzer (Applied Biosystems, Foster City, CA).

## Statistical analysis

Categorical variables were expressed as numbers and percentages, and continuous variables were summarised as mean and SD and as median and minimum–maximum where appropriate. Chi-square test was used to compare categorical variables between the groups. Shapiro–Wilk test was used to test the normal distribution of continuous variables. Student's t-test or Mann–Whitney U test was used for comparison of continuous variables between two groups depending on whether the statistical hypotheses were fulfilled or not. All analyses were performed using IBM SPSS (IBM Corp. Released 2011. IBM SPSS Statistics for Windows, Version 20.0. Armonk, NY: IBM Corp. Statistics Version 20.0 statistical software package). The statistical level of significance for all tests was considered to be 0.05.

# RESULTS

A total of 80 patients (66 women and 14 men) diagnosed with PHPT were included, of which 22 were symptomatic (see detailed analyses in table 1 and 2). Serum calcium and 24-hour calcium excretion were significantly elevated in patients with symptomatic PHTP ($p$ = 0.009, $p$ = 0.00) (Fig 1-2). Serum PTH levels between the two group were

similar ($p$ = 0.667). Bone diseases ($p$ = 0.04) and nephrolithiasis ($p$ = 0.03) were common in patients with symptomatic PHPT with respect to classical manifestations of PHPT. Non-clinical manifestations, for example, fatigue, was present in 77.2% (17/22) patients with symptomatic PHPT. Muscle weakness (68.1% vs 43.1%) and neuropsychiatric symptoms (45.4% vs 17.2%) were also found elevated in patients with symptomatic PHPT. Decision of surgical treatment was positively correlated with elevated serum calcium levels, 24-hour urine calcium levels and decreased serum phosphate levels ($p$ = 0.009, $p$ = 0.00, $p$ = −0.008) (Table 2).

Mean adenoma size of symptomatic and asymptomatic patients was 17 ± 7 mm and 20 ± 11 mm, respectively ($p$ = 0.66). While parathyroid scintigraphy correctly localised in 56 patients (70%), ultrasound as a supplemental study was required for the rest of the patients (n = 24). Ultrasound was performed in six symptomatic PTHP patients whose adenoma localisation could not be corrected by scintigraphy. Mean size of these (n = 6) adenomas was 9 ± 1 mm. The majority of adenomas (n = 40, 50%) were found in right inferior localisation, 20 of them (25%) in left inferior, 12 of them (15%) in right superior and 8 of them (10%) in left superior localisation.

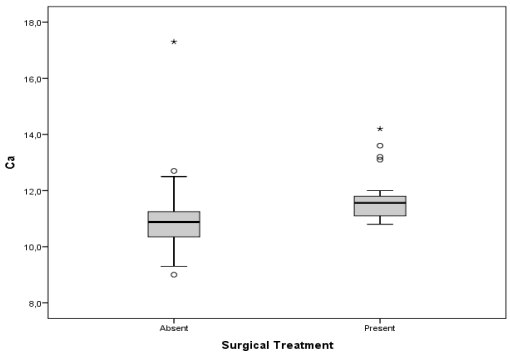


**Figure 1. Correlation between the level of serum calcium and surgical treatment.**

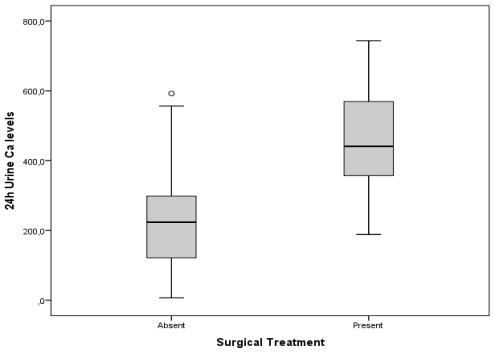


**Figure 2. Correlation between the urine calcium excretion and surgical treatment.**

**Table 1. All patients with PHPT demographical, clinical and biochemical parameters.**

| Variable (referance range) | mean±std. deviation (min-max) |
|---|---|
| Age (y) | 50.8±12.0 (20-75) |
| Calcium (8.9-10.3 mg/dL) | 11.1±1.2 (9-17) |
| Phosphate (2.4-4.7 mg/dL) | 2.6±0.6 (1.3-4.0) |
| PTH (12-88 pg/mL) | 237.9±287.4 (50-2077) |
| BUN (mg/dL) (8-20 mg/dL) | 13.4±7.8 (3-47) |
| Creatinin (0.4-1 mg/dL) | 0.7±0.3 (0.08-2.3) |
| TSH (0.38-5.33 mIU/L) | 2.2±1.8 (0.02-6.49) |
| Urinary Calcium Excretion (100-300 mg/24 h) | 286.9±175.9 (6.5-743.4) |
| Plasma 25-OH vitamin D (10-60 ng/mL) | 25.8±5.76 (20-35) |
| T score LS | -1.2±1.2 |
| T score LH | -1.6±2.2 |
| Nephrolithiasis | 16 (20%) |

PTH: Parathyroid hormone, TSH: Thyroid stimulating hormone, BUN: Blood Urea Nitrogen, LS: Lumbar Spine, LH: Left Hip

**Table 2. Comparison of clinical, biochemical and *CDKN1B* mutation analyses in patients with symptomatic or asymptomatic PHPT.**

| | Symptomatic (n=22) | Asymptomatic (n=58) | p |
|---|---|---|---|
| Age (y) | 47.1±12 | 52.2±11.8 | 0.09 |
| Fatique | 77.2%(n=17) | 34.4%(n=20) | 0.005 |
| Muscle weakness | 68.1%(n=15) | 43.1%(n=25) | 0.034 |
| Neuropsychiatric symptoms | 45.4%(n=10) | 17.2%(n=10) | 0.004 |
| Calcium (8.9-10.3 mg/dL) | 11.7±0.9 | 10.9±1.2 | 0.009 |
| Phosphate (2.4-4.7 mg/dL) | 2.3±0.6 | 2.7±0.5 | 0.008 |
| BUN (mg/dL) (8-20 mg/dL) | 15.5±4.5 | 17.1±6.5 | 0.8 |
| Creatinin (0.4-1 mg/dL) | 0.7±0.4 | 0.5±0.4 | 0.6 |
| PTH (12-88 pg/mL) | 154.6±117.1 | 255.9±333.1 | 0.667 |
| TSH (0.38-5.33 mIU/L) | 2.1±2.3 | 2.4±1.5 | 0.47 |
| Urinary Calcium Excretion (100-300 mg/24 h) | 564.8±169.9 | 309.1±135 | 0.034 |
| T score LS | -2.2±1.4 | -1.3±1.4 | 0.03 |
| T score LH | -2.3±1.3 | -1.2±1.2 | 0.04 |
| Nephrolithiasis | 11 (50%) | 5 (8.6%) | 0.03 |
| Location detected by scintigraphy | 16 | 40 | - |
| Location detected by ultrasound+scintigraphy | 6 | 18 | - |
| Adenoma size (mm) | 17±7 | 20±11 | 0.669 |
| *CDKN1B* | CCTTCC | CCTTCC | - |

PTH: Parathyroid hormone, TSH: Thyroid stimulating hormone, BUN: Blood Urea Nitrogen, LS: Lumbar Spine, LH: Left Hip
Variation ID: rs786201010

When correlation between the serum biochemical parameters (Ca, iPo4, PTH, Cr, 24-hour urine Ca excretion) and corrected adenoma on scintigraphy was analysed, serum calcium levels ($p = 0.01$) and 24-hour urine Ca excretion ($p = 0.003$) were positively correlated with corrected on scintigraphy. Size of adenoma was also positively correlated with corrected on scintigraphy ($p = 0.004$).

CC genotype was detected in all patients with PHPT in rs786201010. c.-456_-453delCCTT was not detected in any patients.

## DISCUSSION

The present study comprising 80 patients with symptomatic and asymptomatic PHPT was compared clinically, biochemically and with respect to genetic mutation *(CDKN1B)* in published literature. A total of 80 patients with PHPT were identified and 22 of them were found symptomatic and the rest were asymptomatic. Symptomatic patients were found to have elevated levels of calcium levels (hypercalcaemic), 24-hour urine calcium excretion and target organ damage (bone disease and nephrolithiasis). Serum PTH level was observed as not a predictive factor to clarify patients with symptomatic or asymptomatic PHPT. Size of adenoma had no effect on the diagnosis of patients with symptomatic or asymptomatic PHPT. However, size of adenoma was positively correlated with scintigraphy. PHPT has a very heterogeneous clinical view and several genes including *CDKN1B* have been implicated in the pathogenesis of sporadic PHPT occurrence. However, in this study findings did not determine deletion mutation in *CDKN1B* gene (rs786201010) in both groups (symptomatic or asymptomatic PHPT).

As we know, it is a common knowledge that most patients with PHPT are asymptomatic and they have neither symptoms nor complications associated with hypercalcaemia and excessive PTH [25,26]. The preponderance of asymptomatic individuals raises important questions such as how to manage of such patients once the diagnosis is established. While some authors described these asymptomatic patients as a new phenotype of PHPT [27], others suggested them as an early form of symptomatic patients [28]. The management and follow-up criteria of asymptomatic

PHPT were expressed at four NIH conferences, one held in 1990 and the others in 2002, 2009 and 2013[29,30]. Consensus of these guidelines recommended detailed evaluations of skeletal and renal involvement for surgery in patients with asymptomatic PHPT. Although asymptomatic PHPT was described as milder presentation of classical PHPT caused by inappropriate secretion or over-secretion of PTH, the exact mechanism of asymptomatic PHPT was not really understood. However, in recently published workshop, it has been reported that there is an association between newly diagnosed PTH adenomas (incidentalomas) and elevated PTH levels [31]. Since neck ultrasound was used more widely, asymptomatic patients were more common in clinical practice. Eufraziano et al. [32] reported 81.8% of patients with PHPT were asymptomatic and the rest (18.2%) were symptomatic. Mean age of asymptomatic patients (61 ± 15 vs 52 ± 18 years) was found to be higher than symptomatic patients in their study. Asymptomatic patients presented lower serum calcium (10.3 ± 0.8 vs 11.7 ± 2.2 mg/dL) and PTH levels (124.2 ± 96.3 vs 444.4 ± 730.28 pg/mL) than symptomatic patients. Classical symptoms including nephrolithiasis, bone diseases and neuropsychiatric were more commonly found in symptomatic patients. In the present study 72.5% of patients were asymptomatic. Serum calcium (10.9 ± 1.2 vs 11.7 ± 0.9 mg/dL) and PTH levels (154.6 ± 117.1 vs 255.9 ± 333.1 pg/mL) of asymptomatic patients were found to be lower than symptomatic patients. Non-specific symptoms (fatigue and muscle weakness) were more commonly found in symptomatic patients. While 24-hour calcium excretion can be used as a clinical marker for renal manifestation, some studies reported 24-hour calcium excretion was lower in asymptomatic group as compared to the symptomatic group [33,34]. Pierreux et al. [35] compared clinical and biochemical parameters of normocalcaemic (n = 25) and hypercalcaemic (n = 106) subjects. Mean calcium levels of normocalcaemic and hypercalcaemic patients were found to be 2.34 ± 0.11 versus 2.71 ± 0.2 mmol/L (reference range 2.10–2.50 mmol/L). However, they reported that 24-hour urine calcium excretion was lower in normocalcaemic subjects as compared to hypercalcaemic group. The present study also shows a similar result. Hypercalciuria likely contributes to an increased risk of renal stones and persistent calciuria even in normocalcaemic patients may cause renal insufficiency. Nephrolithiasis was found to be more common among in patients with symptomatic compared to asymptomatic patients in the present study (50% vs 8.6%, $p$ = 0.03), however, renal insufficiency was not encountered in any of the patients.

Since BMD is an important predictor of fracture risk, the densitometric data in PHPT suggest certain expectations about fracture incidence[36]. In 2016 Castellano[37] studied 172 patients with asymptomatic PHPT and evaluated three sites DEXA scans (forearm, lumbar spine and hip) for surgery. While serum calcium (10.9 ± 0.8 mg/dL) and PTH (123 ng/mL) levels of these patients were seen to be mildly increased, but osteoporosis was found to be more common in three sites DEXA scan (T score: −2.6 ± 4.8, −2.6 ± 3.25, −2.9 ± 2.87, respectively). In our study; DEXA measurements of LH and LS scan were found to be relatively increased in symptomatic patients (T score: -2.3±1.3 and -2.2±1.4) than the asymptomatic patients (T score: -1.2±1.2 and -1.3±1.4) but severe osteoporosis was not observed in any of the patients.

Some patients with asymptomatic PHPT have positive imaging with a parathyroid sestamibi scan because they do not meet surgical treatment criteria [38]. However, some symptomatic patients have negative scans, and then it should be evaluated with detailed screening with the other imaging modalities[39-41]. The indications for surgery are the same for patients with and without localisation on imaging. Studies showed patients with larger parathyroid adenomas (>1.8 cm), and higher preoperative calcium levels are more reliably identified by sestamibi scan [42,43]. In 2014 Wachtel et al. [44]reported that non-localised patients with PHPT had smaller parathyroid adenoma and had lower calcium levels, and they also had a higher incidence of hyperplasia. In this study 56 (70%) of patients had positive localisation on sestamibi scan and the others (n = 24, 30%) were detected by performing a combined ultrasound and scintigraphy. Six of the symptomatic patients who were decided to undergo surgical therapy were negative on scintigraphy, but they were determined with combined ultrasound and scintigraphy. Mean adenoma size of these patients' (9 ± 1 vs 17 ± 7 mm, $p$ = 0.004) of these patients were lower as compared to the rest of symptomatic patients (n=16) with PHPT.

More than 10% of patients with PHPT will have a mutation in one of 11 genes (*Menin, RET, CDKN1B, CDC73, CASR, CDKN1A, CDKN2B, CDKN2C,*

*GNA11, AP2S1*) [45-47]. Clinical evaluation and genetic testing should be carried out when appropriate to define the aetiology of PHPT. With the involvement of *CDKN1B* in familial/syndromic hyperparathyroidism, a role for *CDKN1B* mutation in sporadic parathyroid tumours was sought. Deletion (c.-456_-453delCCTT) in *CDKN1B* was investigated but was not found to be present in any of the 80 patients. The potential role of *CDKN1B* genotyping in parathyroid adenomas can be examined with additional studies of penetrance and occurrence of *de novo* mutations in more patients and extended families of probands with symptomatic and asymptomatic PHPT will be needed.

Overall, the incidence and prevalence of PHPT have increased during the past several decades, even in countries that had established biochemical screening. The two distinct clinical spectrum of primary hyperparathyroidism have become more common. The etiology or clinical management of symptomatic or asymptomatic PHPT had still complicated. Therefore more prospective clinical studies with large sample size are needed.

The strength of the study is the first study in Turkish population in case of mutation analyses in PHPT. Another important issue asymptomatic PHPT is an unknown clinical area and there is limited study up to date. Our study may clarify the clinical, biochemical or molecular variations between these patients with PHPT. Relatively small number of study population is a basic limitation of our study. Another limitation is the evaluation of the restricted area in CDKN1B gene. These limitations can be resolved by further studies.

In summary, asymptomatic hyperparathyroidism has a clinical bias. Patients with asymptomatic hyperparathyroidism have mildly increased calcium levels and 24-hour urine calcium excretion, but target organ manifestations (renal or bone disease) were not common. There were no abnormalities in *CDKN1B*, but the study findings further predict that future studies of sporadic hyperparathyroidism will reveal additional examples in *CDKN1B* and/or other genes.

**Yazar Katkıları:** Çalışma konsepti/Tasarımı: GA ; Veri toplama:SC, BK ; Veri analizi ve yorumlama: GA,SC,BK ; Yazı taslağı: GA; İçeriğin eleştirel incelenmesi TT: NÖM; Son onay ve sorumluluk GA:; Teknik ve malzeme desteği: -; Süpervizyon:GA,TT ; Fon sağlama (mevcut ise): yok.
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**Author Contributions:** Concept/Design : ; Data acquisition: ; Data analysis and interpretation: ; Drafting manuscript: ; Critical revision of manuscript: ; Final approval and accountability: ; Technical or material support: -; Supervision: ; Securing funding (if available): n/a.
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