Original Article

Clinicopathological Profile of Primary Hyperparathyroidism with Special Reference to Ki-67 Labelling Index

Azhar S. Thanveer, Sadishkumar Kamalanathan[1], Bhawana A. Badhe, Rajan Palui[1], Kengunte G. Rashmi[1], Naadia F. Nadeem

Departments of Pathology and [1]Endocrinology, Jawaharlal Institute of Postgraduate Medical Education and Research (JIPMER), Puducherry, India

Abstract

Context: Primary hyperparathyroidism (PHPT) can occur due to a neoplastic process or hyperplasia. While the disease presentation is predominantly asymptomatic in developed countries, this is not the case yet in India. Differentiation of the type of lesion can only be done based on histomorphology but has its own challenges. Immunohistochemical markers like Ki-67 have been studied to aid in diagnosis but data on this is sparse from India. **Aims:** The aim of this study is to assess the clinical, biochemical and pathological profile of PHPT and to analyse the differences in immunohistochemical marker Ki-67 among the various lesions. **Setting and Design:** A descriptive study was carried out on 38 PHPT patients who were treated at our institute from January 2011 to March 2021. **Materials and Methods:** Post-surgery, the causative lesions were categorised as adenoma (31), hyperplasia (5) and carcinoma (2). Clinical, biochemical, radiological and histopathological features of all lesions were collected and analysed. Ki-67 proliferation index was calculated. The various parameters were compared across the three groups of lesions and correlated with Ki-67 index. **Results:** Out of 38 patients, 37 were symptomatic with skeletal symptoms being the most common followed by renal symptoms. There was no difference in clinical or biochemical parameters among the three types of lesions. Significant negative correlation was seen between serum iPTH and serum 25-OH Vitamin D levels (*P0.006)* The median Ki-67 index was found to be 0.40% in hyperplasia, 0.49% in adenoma and 5.84% in carcinoma. **Conclusion:** PHPT still presents as an overtly symptomatic disease in India. Diagnosis of the nature of lesion depends on the accurate application of morphological criteria. A high Ki-67 index was not found to be an absolute marker of carcinoma, as it was also seen in a small proportion of atypical adenomas.

Keywords: Immunohistochemistry, Ki-67 index, parathyroid adenoma, parathyroid carcinoma, primary hyperparathyroidism

Introduction

Primary hyperparathyroidism (PHPT), the most common cause of hypercalcemia,[1] is characterised by overproduction of parathormone (PTH) leading to derangement of calcium homeostasis. Such an increase in PTH secretion is most commonly due to an adenoma, and less frequently due to hyperplasia or rarely carcinoma.[2,3]

A varied clinical profile of this disease is seen in different geographical regions with the asymptomatic variety most prevalent in developed countries. In India, however it is still seen with classic manifestations involving the musculoskeletal, renal and gastrointestinal systems.[4,5] The high prevalence of vitamin D deficiency in the Indian population is an added feature which can significantly affect both the clinical presentation as well as management.[6-10]

While the diagnosis of PHPT can be made easily, differentiation between the three types of lesions can be challenging.[11,12] Furthermore, certain benign lesions, described as atypical adenomas, can present with several features classically associated with malignancy and hence can be difficult to classify and categorise.[13] Several molecular markers have been studied to aid in diagnosis, of which the nuclear proliferation marker, Ki-67 shows particularly favourable results in identifying carcinomas.[14,15] There is however a paucity of data on its use especially from India.

Access this article online

Quick Response Code:

Website: www.ijem.in

DOI: 10.4103/ijem.ijem_208_22

Address for correspondence: Dr. Sadishkumar Kamalanathan, Department of Endocrinology, Jawaharlal Institute of Postgraduate Medical Education and Research (JIPMER), Dhanvantri Nagar, Puducherry, India.
E-mail: sadishkk@gmail.com

Submitted: 23-May-2022 **Published:** 20-Dec-2022
Accepted: 21-Jul-2022

How to cite this article: Thanveer AS, Kamalanathan S, Badhe BA, Palui R, Rashmi KG, Nadeem NF. Clinicopathological profile of primary hyperparathyroidism with special reference to Ki-67 labelling index. Indian J Endocr Metab 2023;27:73-9.

Our study was designed with the objective to assess the clinical, biochemical, hormonal and pathological profile of patients presenting with PHPT at a tertiary care centre in South India and to analyse the use of Ki-67 as a definite marker for identifying malignancy.

Materials and Methods

Patient selection

The study included 38 patients who were treated for PHPT at our institute, from January 2011 to March 2021 which can be taken as representative of the South Indian population due to our centre being a tertiary care referral centre. Those patients whose surgically resected parathyroid glands were not subjected to histopathology at our centre were excluded.

Clinical data collection

The archived medical case records of all the patients were studied. Clinical symptoms were recorded and categorised as skeletal, renal, gastrointestinal, neuromuscular and neuropsychiatric symptoms. Data on biochemical parameters and radiological investigations were obtained from case records as well as from the Hospital Information System and the Picture Archiving and Communication System at the institute. Serum calcium (Ca), intact parathormone (iPTH), phosphate, albumin, alkaline phosphatase (ALP) and 25-OH vitamin D at the time of diagnosis were compiled. Immediate post-ligation iPTH values (intra-operative) and post-operative serum Ca values were recorded. Data on effective localisation of the lesion using ultrasonogram (USG), ^{99m}Technetium-sestamibi (MIBI) scan was also assessed. Bone mineral density (BMD) at lumbar spine (total), distal 1/3rd of radius and neck of femur were evaluated by Dual energy X-ray absorptiometry (DXA) scan (Hologic Inc., USA).

Histopathological data collection

The paraffin blocks of surgically resected specimens were retrieved from the archives of the Department of Pathology. Morphology of the tumour was studied by preparing 3 μm Haematoxylin and Eosin (H&E) sections.

For preparing slides for Ki-67 immunostaining, deep section of the tissue at 1000 μm was taken. Staining for Ki-67 was done using mouse monoclonal MIB-1 antibodies and secondary staining with Horseradish Peroxidase and 3,3'-Diaminobenzidine using Polymer Envision kit (all supplied by Dako., Santa Clara, CA, USA). The staining procedure was done according to the protocol followed in our lab with the duration of heat-based antigen retrieval increased to 90 minutes considering the age of blocks used.[16]

The stained slides were screened to look for areas of highest uptake and the Ki-67 proliferation was calculated by counting the number of positively stained nuclei from images captured using the microscope. A minimum of 500 cells were counted in each case and the final Ki-67 score was expressed as a percentage. Ki-67 in the rim of normal parathyroid surrounding lesions was also calculated. This method was carried out by two of the authors to reduce error.

Statistical analysis

All data collected was analysed using IBM SPSS Statistics software version 19.0 (IBM, USA 2018). The results were expressed as percentages for prevalence of clinical manifestations. Biochemical parameters were summarised as mean and standard deviation. Histological characteristics were summarised as proportions. Ki-67 index across the groups was expressed as median and range. Comparison of qualitative variables across the three types of lesions was done using Chi-square test. Biochemical parameters of adenoma, hyperplasia and carcinoma were compared using Kruskal–Wallis test. Comparison of Ki-67 among the groups was also done by Kruskal–Wallis test. Correlation among the biochemical variables was studied using Pearson's correlation and that between Ki-67 and biochemical parameters was analysed by Spearman's rank correlation.

Statement on ethics

The study was conducted after obtaining approval from the Institutional Ethics Committee (IEC). All the procedures followed were in accordance with the ethical standards of the IEC and the revised Helsinki Declaration of 2000. All steps were taken to ensure confidentiality of subjects was maintained and no data had any obvious personal identifiers.

Results

Demographic details and clinical presentation

The cases were categorised as adenoma (31), hyperplasia (5) and carcinoma (2) based on current World Health Organisation criteria.[17] Of the 31 adenomas, 2 (6.5%) were atypical adenomas.

The mean age of presentation of the patients was 47 years (range: 19-72 years). In our cohort of patients, a slight female preponderance was seen (1:1.71). Skeletal (78.9%) and renal (57.9%) symptoms were the most common at presentation, followed by gastrointestinal (55.3%) and muscular (44.7%) symptoms. Two (5.26%) of the patients had prostatic calcification. Only one (2.63%) patient was completely asymptomatic. None of the patients had MEN syndromes. The clinical features are summarised in Table 1. There was no difference in clinical symptoms across the different types of lesions.

Biochemical parameters

Biochemical variables analysed are described in Table 2 along with a comparison with similar studies. All patients had elevated calcium levels at diagnosis and 12 patients were found to have high post-operative serum Ca. One patient had normal corrected serum Ca at diagnosis. Mean pre-operative corrected serum Ca was 12.71 mg/dL (S.D 1.71). Mean post-operative serum Ca was 8.72 mg/dL (S.D 1.03) and median post-operative iPTH level was 25.1 pg/mL (inter-quartile range 12.6-69).

Among those with available data, 24 (72.7%) patients had vitamin D deficiency (25-OH vitamin D level <20 ng/mL) with 12 (36.3%) patients having vitamin D levels ≤10 ng/mL. Serum PTH levels were found to have statistically significant *(P 0.006)* negative correlation with serum 25-OH vitamin D levels.

Radiological investigations

Out of the data available for 35 patients, the tumour was localised in 29 (82.9%) patients by USG. In five cases where USG had failed to detect the lesion, localisation could be done by MIBI scan. MIBI scan could detect the lesion in 34 out of 37 (91.9%) patients. In one of the patients, USG was successful in identifying the lesion which could not be detected by MIBI scan. In this case, confirmation was done using 4-dimensional computed tomography (4DCT) scan of the neck. In one of the patients who had coexistent multinodular goiter, the lesion could not be localised by USG, MIBI or CECT scan and the lesion was identified only after thyroidectomy.

Table 1: Clinical manifestations (*n*=38)

Clinical Features*	Prevalence (in %)
Renal	
Nephrolithiasis	34.2
Nephrocalcinosis	34.2
Skeletal	
Osteoporosis	50
Bone/Joint pain	47.4
Fractures	7.9
Brown tumour	13.2
Muscular	
Muscle weakness	28.9
Myalgia	23.7
Gastrointestinal	
Abdominal pain	44.7
Chronic pancreatitis	26.3
Constipation	13.2
Other	
Polyuria	10.5
Neuropsychiatric manifestations	5.3
Anorexia	21.1
Neck mass	10.5

*Multiple responses considered

Of the 38 patients sampled, 32 had lesions involving a single gland only. Twelve (31.6%) lesions involved the right inferior gland whereas nine (23.7%) involved the left inferior gland. Left superior gland was involved in eight (21.1%) cases while the right superior gland was involved in only three (7.9%) cases.

Two lesions were detected as double adenomas involving both left glands in one case and the left-superior and right-inferior glands in the other. One case of hyperplasia showed involvement of both the left glands while another showed involvement of all four glands. One of the adenomas was large and cystic present along the left inferior pole of thyroid while another was ectopically located in the right tracheoesophageal groove posterior to the carotid artery.

Pathological findings

Distribution of the pathological characteristics is given in Table 3. The median weight of the removed parathyroid gland was 4 g (range 1-27 g). The colour of the gland was highly variable but greyish brown to grey white were the most common. All neoplastic lesions were encapsulated. Lobular pattern of arrangement was the most common type among adenomas (83.9%). Six lesions, including both carcinomas, had cells arranged in sheets. One of the adenomas had a bizarre arrangement showing cells in a vague nesting pattern while, one of the hyperplastic parathyroids had a glandular pattern of arrangement. Chief cells were the predominant type of cells in all three lesions. Two adenomas showed oxyphil cells as the predominant cell type.

One of the adenomas showed areas of fibrosis while another had areas of hyalinisation with dystrophic calcification. Both cases of carcinoma showed broad fibrous bands. Two were atypical adenomas showing thick fibrous bands, pleomorphic cells, nuclear atypia and high mitotic figures. Figure 1 demonstrates the characteristic pathological features of the various lesions. Capsular invasion was seen in both carcinomas and vascular invasion in one.

Ki-67 index

The Ki-67 index of all the lesions are summarised in Table 4. The normal parathyroid rim, wherever present surrounding the tumours, showed no Ki-67 reactivity (0%). Twenty-one adenomas showed proliferation index <1% while five

Table 2: Biochemical parameters with comparison to similar studies

Parameter	Present study (*n*=38)	Jacob *et al*.[18] (*n*=88)	Gopal *et al*.[19] (*n*=79)	Pradeep *et al*.[2] (*n*=100)	Misgar *et al*.[20] (*n*=78)	Bhadada *et al*.[21] (*n*=464)
Year	2021	2006	2010	2011	2016	2017
Region	Puducherry	Vellore	Mumbai	Lucknow	Srinagar	Varied
Serum Ca	12.63±1.5 mg/dL	2.97±0.25 mmol/L	12.55±1.77 mg/dL	3.14±0.41 mmol/L	12.5±1.7 mg/dL	11.9±1.6 mg/dL
Serum iPTH (pg/mL)	794.75±722.89	623±714	866.61±799.15	1005.8±760.3	377.6±386.1	752.4±735.2
Serum 25OH- vitamin D (ng/mL)	15.53±10.56	N. A	N. A	11.6±8.74	37.2±58.1	22.9±25.1
Serum ALP (IU/L)	628.8±852.18	426±549	762.2±754.8	1466.5±1547.6	255.1±336.8	653±1180
Serum Phosphate (mg/dL)	2.85±0.91	0.74±0.18	1.81±0.68	N. A	2.2±0.6	2.8±0.9

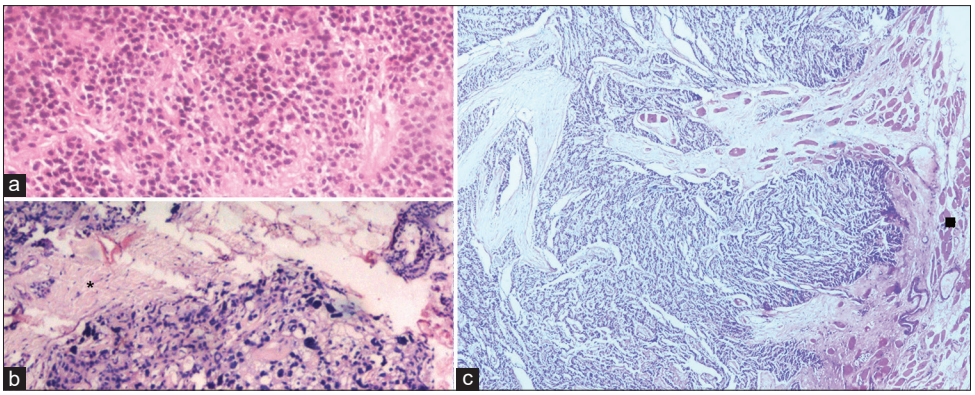

Figure 1: Comparison of typical adenoma (a) showing chief cells with monomorphic nuclei and absence of fat; atypical adenoma. (b) with bizarre cells, prominent nuclear atypia, fibrous bands (*) and carcinoma. (c) showing invasion of tumour cells beyond the capsule into surrounding skeletal muscle (■)

adenomas showed a proliferation index greater than 1%. 2 adenomas had high Ki-67 index more than 4%. Five adenomas showed no positive staining with Ki-67 (proliferation index 0%). All hyperplasias showed a Ki-67 proliferation index ≤1%. Both carcinomas showed a Ki-67 proliferation index >3%. Figure 2 shows Ki-67 staining of adenoma and carcinoma. There was no statistically significant difference in values between the three groups *(P 0.084)*. However, on separating the atypical adenomas as a separate group, there was a statistically significant difference between the groups *(P 0.014)*. No significant correlation was found between Ki-67 and other biochemical parameters.

Discussion

The study was done to find out the clinical picture of patients with PHPT, presenting to our centre and to correlate them with the tumour pathology. As ours is a tertiary care centre catering to patients from all over South India, the sample could be taken to represent the entire region. The mean age of presentation was 47 ± 13 years which is similar to that published in recent Indian studies[20-22] but higher than certain older studies.[19,23,24] The mean age at presentation was also found to increase over the years in a study carried out by Shah *et al*.[25] The disease also shows preponderance towards females irrespective of geography.

It was seen that almost all patients had overtly symptomatic disease which is in agreement with other recent literature from India.[2,21,25,26] The symptomatic presentation has most commonly been attributed to the lack of routine biochemical screening of patients.[27] Only one of the patients had none of the classical symptoms of PHPT in our study. Asymptomatic PHPT in India has been reported to be seen in 5% of patients in a study conducted by Bhadada *et al*.[21] while another study by Mithal *et al*.[22] places this number at 38%. There has also been an increase in the proportion of asymptomatic PHPT in India[28] and a study by Yadav *et al*.[5] also suggests a trend towards milder forms of the disease in the developing world.

Table 3: Pathological features of three types of lesions

Parameter	Adenoma (*n*=31)	Hyperplasia (*n*=5)	Carcinoma (*n*=2)
Size (average) (in cm)	2.5 x 1.6 x 1.9	2.7 x 1.6 x 0.8	1.6 x 1.3 x 0.3
Nodular appearance	16 (51.6%)	1 (20%)	1 (50%)
Cystic change	2 (6.5%)	-	-
Consistency			
Firm/hard	23 (74.2%)	2 (40%)	2 (100%)
Soft/fatty	1 (3.2%)	1 (20%)	-
Haemorrhage	5 (16.1%)	-	1 (50%)
Necrosis	-	-	1 (50%)
Encapsulation	31 (100%)	4 (80%)	2 (100%)
Normal parathyroid rim	20 (64.5%)	3 (60%)	1 (50%)
Fat in lesion	6 (19.4%)	2 (40%)	1 (50%)
Pleomorphic cells	14 (45.2%)	1 (20%)	2 (100%)
Nuclear atypia	10 (32.3%)	1 (20%)	2 (100%)

Table 4: Comparison* of Ki-67 index (as %) among the four types of lesion**

Category of lesion	Number	Minimum	Maximum	Median
Adenoma (typical)	29	0.00	1.86	0.48
Hyperplasia	5	0.17	1.00	0.40
Atypical adenoma	2	4.21	5.64	4.93
Carcinoma	2	3.90	7.78	5.84

*Done by Kruskal–Wallis test **P=0.014

Symptoms involving the skeletal, renal systems along with muscle weakness or fatigability have been reported as the most common symptoms from India.[2,21] Our study had similar findings with skeletal (79%) and renal symptoms (58%) predominating. Also, there is no difference in clinical features among the three different types of lesions.[29]

In India, most cases present with hypercalcemia and high PTH levels. Normocalcemic PHPT is however seen rarely and just one of our patients had a normal corrected serum Ca at diagnosis.

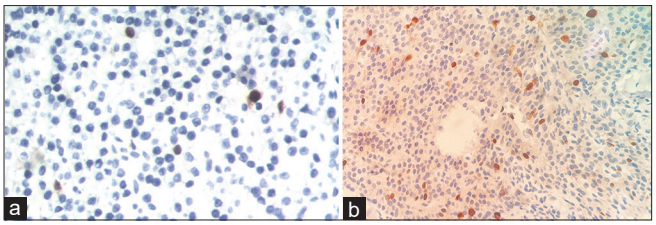

Figure 2: Parathyroid adenoma (a) showing Low Ki-67 proliferation index <1% and carcinoma. (b) showing high Ki-67 proliferation index >5%

BMD measurement by DXA is also currently recommended to assess the severity of metabolic bone disease to predict fracture risk and aid in better management. Both vitamin D and PTH have a tendency to decrease bone mineral density and a positive correlation was found between serum PTH levels and T-scores (total hip) (*P 0.013*). Thus, BMD evaluation is especially necessary in India where vitamin D deficiency can further deteriorate the condition.[6-9] We also found a significant inverse relationship between serum vitamin D levels and serum PTH levels. While there are several mechanisms to explain this, an important clinical implication is in there being a higher chance of severe bone-related symptoms in patients with severe disease.[3]

For preoperative localisation of tumours, USG appears to be a cheap alternative. However, ^{99m}Technitium-sestamibi (MIBI) scan remains the gold standard. Single-photon emission computed tomography-computed tomography (SPECT)/CT scan can be used in cases where disparities exist. Newer modalities like 4-dimensional computed tomography (4DCT) and 18 F-fluorocholine positron emission tomography (FCH PET/CT) scans can be alternatives in such cases if available.[30] A simple technique like USG if performed by an experienced sonographer can be an effective first line strategy in pre-operative localisation of lesions.[31]

Surgery has been indicated in all cases of symptomatic disease and is the definitive form of treatment.[32] All patients in the series were managed surgically. For those patients with available data, all showed a drop in immediate post-operative iPTH levels as assessed by Miami criteria.[33] Local recurrence was seen in only one case of carcinoma and repeat surgery was done to remove the tumour.

In our study, adenoma was diagnosed in 81.6%, hyperplasia in 13.1% and carcinoma in 5.3%. This is similar to other studies across India where the incidence of adenoma ranges from 85 to 95% and carcinoma is only 1-5%.[2,21] The relatively high incidence of carcinomas in the study could be due to the sampling being done from a tertiary referral care setup and the small sample size.

The parathyroid gland has always placed challenges to the pathologist. Differentiation between the three types of lesions requires considerations of several parameters. Parathyroid adenomas classically present as single lesions; however, double or even multiple adenomas may be seen.[34,35] Most are solid and nodular or bosselated but may rarely have areas of cystic degeneration. Characteristic features of adenomas include encapsulation, thin rim of compressed normal parathyroid, lack of stromal fat and predominantly single-cell type, mostly chief cells. Variable architectural patterns may be noted such as cords, nests, sheets, etc.[35] Water-clear cells, oxyphil adenomas (>90% oxyphil cells), lipoadenomas and oncocytic variants can also be seen, though rare.[34,36] Mild nuclear atypia may also be seen in benign lesions.

Unlike parathyroid adenomas, hyperplasia is a multiglandular process and to confirm the diagnosis demonstration of involvement of all glands is needed.[32] These lesions show stromal fat, although, it is reduced. The cell type is mostly mixed, containing chief, oxyphil and water-clear cells. Secondary and tertiary causes of PHPT must also be clinically eliminated to diagnose primary parathyroid hyperplasia.[34,35]

Diagnosis of parathyroid carcinoma is always challenging if clinical evidence of metastasis or gross local invasion is not present.[11] The histological features which are definite indicators of malignancy include capsular invasion with growth into surrounding soft tissue, vascular invasion and perineural invasion. Even these features can be subjective to assess and may not even be present in all carcinomas.[37] Capsular invasion in tumours with thick capsule where pseudo-invasion may be seen can be difficult to differentiate. Figure 2 shows the characteristic capsular invasion of carcinomas.

Features like thick fibrous bands traversing through the lesion, high degree of nuclear pleomorphism, high mitotic index, necrosis, macro-nucleoli and giant cells are said to be suggestive of malignancy but they can be seen in benign lesions as well.[12,38] Such lesions, have been described as atypical adenomas[13] or minimally invasive parathyroid carcinomas[39,40] and can be difficult to categorise. Thus, only features of invasion or metastasis remain absolute indicators of malignancy. It should be noted that the presence of mitosis (>5/50 HPF), macro-nucleoli and presence of necrosis are poor prognostic features with higher chances of recurrent disease.[11,34-37]

The diagnosis of the type of lesion can be simplified to an extent by the use of simple immunohistochemical techniques. Nuclear proliferation markers Ki-67 and Cyclin D1 have shown favorable results.[11,37] We assessed the role of Ki-67 and found that while all carcinomas had a high Ki-67 proliferation index, this was not exclusive to carcinomas. Atypical adenomas can have a high Ki-67 index which precludes the use of Ki-67 as a definite marker of malignancy and restrict its use to being a negative marker of malignancy at the most. A comparison of Ki-67 with other studies is given in Table 5.

The findings of our study have shown results similar to another study from India conducted by Kumari *et al*.[42] However, a clear cutoff for Ki-67, suggested in studies[49] as >5% has not been shown which can aid definitive diagnosis. Other markers which have been studied include loss of parafibromin expression in carcinomas.[49-51] bcl-2,

Table 5: Comparison of Ki-67 index among studies

Study	Region	Year	Ki-67 index (as %)		
			Hyperplasia	Adenoma	Carcinoma
Present study	India	2017	0.4	0.49	5.84
Abbona *et al*.[14]	Italy	1995	2.6	3.28	7.86
Karak *et al*.[41]	India	1997	1.17	1.36	N. A
Kumari *et al*.[42]	India	2016	N. A	0.4	6.7
Lloyd *et al*.[43]	United States	1995	2.7	2.4	7.1
Lumachi *et al*.[44]	Italy	2006	N. A	N. A	13.9
Saggiorato *et al*.[45]	Italy	2006	0.23	1.92	6.75
Vargas *et al*.[46]	United States	1997	N. A	2.03	7.98
Kaczmarek *et al*.[47]	Poland	2008	1.8	1.9	N. A
Kameyama *et al*.[48]	Japan	2000	N. A	N. A	9.9

p27, galectin-3 and tissue microarrays have also been suggested to help identify the type of lesion.[37,42,50] The use of such techniques also face the additional challenge of availability and cost especially in resource-poor settings. Due to the inherent rarity of the disease, a multi-centre trial from India, which has a significantly different disease profile, would help towards improving the diagnosis of the lesion. This would also have the added advantage of uniformity of methods and techniques.

Limitations

A major limitation in our study was the low number of participants, with a relatively higher percentage of carcinoma and atypical adenomas. Nevertheless, their numbers were small, making it difficult to make significant comparison. As ours was a retrospective study on archived data, there was also an issue of missed data in case of certain variables.

Conclusion

PHPT is still diagnosed in India at a stage where the disease has progressed to produce florid symptoms. Histopathological criteria still remain paramount in the diagnosis of the type of lesion. The nuclear proliferation marker Ki-67 shows particular promise but the entity of atypical adenoma showing severe atypia and high proliferation index cannot be distinguished from malignant lesions using this marker.

Acknowledgements

The authors would like to acknowledge Dr. Rajeswari Murugesan, department of Biostatistics for the contribution towards statistical methods and analysis; Mrs. Kalaivizhi and Mrs. Girija from the Department of Pathology for their technical assistance in histopathology and immunohistochemistry laboratories.

Financial support and sponsorship

This study was supported by the JIPMER Golden Jubilee GJ-STRAUS intramural research grant.

Conflicts of interest

There are no conflicts of interest.

References

1. AACE/AAES Task Force on Primary Hyperparathyroidism. The American Association of Clinical Endocrinologists and the American Association of Endocrine Surgeons position statement on the diagnosis and management of primary hyperparathyroidism. Endocr Pract 2005;11:49-54.
2. Pradeep PV, Jayashree B, Mishra A, Mishra SK. Systematic review of primary hyperparathyroidism in India: The past, present, and the future trends. Int J Endocrinol 2011;2011:921814.
3. Walker MD, Silverberg SJ. Primary hyperparathyroidism. Nat Rev Endocrinol 2018;14:115-25.
4. Khan AA, Hanley DA, Rizzoli R, Bollerslev J, Young JE, Rejnmark L, *et al*. Primary hyperparathyroidism: Review and recommendations on evaluation, diagnosis, and management. A Canadian and international consensus. Osteoporos Int 2017;28:1-19.
5. Yadav SK, Johri G, Bichoo RA, Jha CK, Kintu-Luwaga R, Mishra SK. Primary hyperparathyroidism in developing world: A systematic review on the changing clinical profile of the disease. Arch Endocrinol Metab 2020;64:105-10.
6. Moosgaard B, Christensen SE, Vestergaard P, Heickendorff L, Christiansen P, Mosekilde L. Vitamin D metabolites and skeletal consequences in primary hyperparathyroidism. Clin Endocrinol (Oxf) 2008;68:707-15.
7. Pradeep PV, Mishra A, Agarwal G, Agarwal A, Verma AK, Mishra SK. Long-term outcome after parathyroidectomy in patients with advanced primary hyperparathyroidism and associated vitamin D deficiency. World J Surg 2008;32:829-35.
8. Agarwal A, Gupta SK, Sukumar R. Hyperparathyroidism and malnutrition with severe vitamin D deficiency. World J Surg 2009;33:2303-13.
9. Silverberg SJ, Shane E, Dempster DW, Bilezikian JP. The effects of vitamin D insufficiency in patients with primary hyperparathyroidism. Am J Med 1999;107:561-7.
10. Harinarayan CV, Gupta N, Kochupillai N. Vitamin D status in primary hyperparathyroidism in India. Clin Endocrinol (Oxf) 1995;43:351-8.
11. Cetani F, Pardi E, Marcocci C. Update on parathyroid carcinoma. J Endocrinol Invest 2016;39:595-606.
12. Guilmette J, Sadow PM. Parathyroid pathology. Surg Pathol Clin 2019;12:1007-19.
13. Galani A, Morandi R, Dimko M, Molfino S, Baronchelli C, Lai S, *et al*. Atypical parathyroid adenoma: Clinical and anatomical pathologic features. World J Surg Oncol 2021;19:19.
14. Abbona GC, Papotti M, Gasparri G, Bussolati G. Proliferative activity in parathyroid tumors as detected by Ki-67 immunostaining. Hum Pathol 1995;26:135-8.
15. Erickson LA, Mete O. Immunohistochemistry in diagnostic parathyroid pathology. Endocr Pathol 2018;29:113-29.
16. Grillo F, Bruzzone M, Pigozzi S, Prosapio S, Migliora P, Fiocca R, *et al*. Immunohistochemistry on old archival paraffin blocks: Is there an expiry date? J Clin Pathol 2017;70:988-93.

17. DeLellis RA, Arnold A, Bilezikian JP, Eng C, Erickson LA, Franssila KO, *et al*. Tumours of the parathyroid glands. In: Lloyd RV, Osamura RY, Klöppel G, Rosai J, editors. WHO Classification of Tumours of Endocrine Organs. 4th ed. Lyon, France: International Agency for Research on Cancer (IARC) Press; 2017. p. 145-59.
18. Jacob JJ, John M, Thomas N, Chacko A, Cherian R, Selvan B, *et al*. Does hyperparathyroidism cause pancreatitis? A South Indian experience and a review of published work. ANZ J Surg 2006;76:740-4.
19. Gopal RA, Acharya SV, Bandgar T, Menon PS, Dalvi AN, Shah NS. Clinical profile of primary hyperparathyroidism from western India: A single center experience. J Postgrad Med 2010;56:79-84.
20. Misgar RA, Dar PM, Masoodi SR, Ahmad M, Wani KA, Wani AI, *et al*. Clinical and laboratory profile of primary hyperparathyroidism in Kashmir Valley: A single-center experience. Indian J Endocrinol Metab 2016;20:696-701.
21. Bhadada SK, Arya AK, Mukhopadhyay S, Khadgawat R, Sukumar S, Lodha S, *et al*. Primary hyperparathyroidism: Insights from the Indian PHPT registry. J Bone Miner Metab 2018;36:238-45.
22. Mithal A, Kaur P, Singh VP, Sarin D, Rao DS. Asymptomatic primary hyperparathyroidism exists in North India: Retrospective data from 2 tertiary care centers. Endocr Pract 2015;21:581-5.
23. Priya G, Jyotsna VP, Gupta N, Chumber S, Bal CS, Karak AK, *et al*. Clinical and laboratory profile of primary hyperparathyroidism in India. Postgrad Med J 2008;84:34-9.
24. Mishra SK, Agarwal G, Kar DK, Gupta SK, Mithal A, Rastad J. Unique clinical characteristics of primary hyperparathyroidism in India. Br J Surg 2001;88:708-14.
25. Shah VN, Bhadada S, Bhansali A, Behera A, Mittal BR. Changes in clinical & biochemical presentations of primary hyperparathyroidism in India over a period of 20 years. Indian J Med Res 2014;139:694-9.
26. Jha S, Jayaraman M, Jha A, Jha R, Modi KD, Kelwadee JV. Primary hyperparathyroidism: A changing scenario in India. Indian J Endocrinol Metab 2016;20:80-3.
27. Bandeira L, Bilezikian J. Primary Hyperparathyroidism. F1000Res 2016;5:1.
28. Arya AK, Kumari P, Bhadada SK, Agrawal K, Singh P, Mukherjee S, *et al*. Progressive rise in the prevalence of asymptomatic primary hyperparathyroidism in India: Data from PHPT registry. J Bone Miner Metab 2021;39:253-9.
29. Agarwal G, Prasad KK, Kar DK, Krishnani N, Pandey R, Mishra SK. Indian primary hyperparathyroidism patients with parathyroid carcinoma do not differ in clinicoinvestigative characteristics from those with benign parathyroid pathology. World J Surg 2006;30:732-42.
30. Thanseer N, Bhadada SK, Sood A, Mittal BR, Behera A, Gorla AKR, *et al*. Comparative effectiveness of ultrasonography, 99mTc-sestamibi, and 18F-fluorocholine PET/CT in detecting parathyroid adenomas in patients with primary hyperparathyroidism. Clin Nucl Med 2017;42:e491-7.
31. Treglia G, Trimboli P, Huellner M, Giovanella L. Imaging in primary hyperparathyroidism: Focus on the evidence-based diagnostic performance of different methods. Minerva Endocrinol 2018;43:133-43.
32. Bilezikian JP, Cusano NE, Khan AA, Liu JM, Marcocci C, Bandeira F. Primary hyperparathyroidism. Nat Rev Dis Primers 2016;2:16033.
33. Carneiro DM, Solorzano CC, Nader MC, Ramirez M, Irvin GL. Comparison of intraoperative iPTH assay (QPTH) criteria in guiding parathyroidectomy: Which criterion is the most accurate? Surgery 2003;134:973-9.
34. Johnson SJ. Changing clinicopathological practice in parathyroid disease. Histopathology 2010;56:835-51.
35. Duan K, Gomez Hernandez K, Mete O. Clinicopathological correlates of hyperparathyroidism. J Clin Pathol 2015;68:771-87.
36. Carlson D. Parathyroid pathology: Hyperparathyroidism and parathyroid tumors. Arch Pathol Lab Med 2010;134:1639-44.
37. Stojadinovic A, Hoos A, Nissan A, Dudas ME, Cordon-Cardo C, Shaha AR, *et al*. Parathyroid neoplasms: Clinical, histopathological, and tissue microarray-based molecular analysis. Hum Pathol 2003;34:54-64.
38. Schulte JJ, Pease G, Taxy JB, Hall C, Cipriani NA. Distinguishing parathyromatosis, atypical parathyroid adenomas, and parathyroid carcinomas utilizing histologic and clinical features. Head Neck Pathol 2021;15:727-36.
39. Singh P, Vadi SK, Saikia UN, Sood A, Dahiya D, Arya AK, *et al*. Minimally invasive parathyroid carcinoma-A missing entity between parathyroid adenoma and carcinoma: Scintigraphic and histological features. Clin Endocrinol (Oxf) 2019;91:842-50.
40. Kameyama K, Takami H. Proposal for the histological classification of parathyroid carcinoma. Endocr Pathol 2005;16:49-52.
41. Karak AK, Sarkar C, Chumber S, Tandon N. MIB-1 proliferative index in parathyroid adenoma & hyperplasia. Indian J Med Res 1997;105:235-8.
42. Kumari N, Chaudhary N, Pradhan R, Agarwal A, Krishnani N. Role of histological criteria and immunohistochemical markers in predicting risk of malignancy in parathyroid neoplasms. Endocr Pathol 2016;27:87-96.
43. Lloyd RV, Carney JA, Ferreiro JA, Jin L, Thompson GB, Van Heerden JA, *et al*. Immunohistochemical analysis of the cell cycle-associated antigens Ki-67 and retinoblastoma protein in parathyroid carcinomas and adenomas. Endocr Pathol 1995;6:279-87.
44. Lumachi F, Ermani M, Marino F, Iacobone M, Baldessin M, Cappuzzo G, *et al*. PCNA-LII, Ki-67 immunostaining, p53 activity and histopathological variables in predicting the clinical outcome in patients with parathyroid carcinoma. Anticancer Res 2006;26:1305-8.
45. Saggiorato E, Bergero N, Volante M, Bacillo E, Rosas R, Gasparri G, *et al*. Galectin-3 and Ki-67 expression in multiglandular parathyroid lesions. Am J Clin Pathol 2006;126:59-66.
46. Vargas MP, Vargas HI, Kleiner DE, Merino MJ. The role of prognostic markers (MiB-1, RB, and bcl-2) in the diagnosis of parathyroid tumors. Mod Pathol 1997;10:12-7.
47. Kaczmarek E, Lacka K, Majewski P, Trzeciak P, Gorna A, Jarmolowska-Jurczyszyn D, *et al*. Selected markers of proliferation and apoptosis in the parathyroid lesions: A spatial visualization and quantification. J Mol Histol 2008;39:509-17.
48. Kameyama K, Takami H, Umemura S, Osamura YR, Wada N, Sugino K, *et al*. PCNA and Ki-67 as prognostic markers in human parathyroid carcinomas. Ann Surg Oncol 2000;7:301-4.
49. Duan K, Mete Ö. Parathyroid carcinoma: Diagnosis and clinical implications. Turk Patoloji Derg 2015;31:80-97.
50. Fernandez-Ranvier GG, Khanafshar E, Tacha D, Wong M, Kebebew E, Duh QY, *et al*. Defining a molecular phenotype for benign and malignant parathyroid tumors. Cancer 2009;115:334-44.
51. Hosny Mohammed K, Siddiqui MT, Willis BC, Zaharieva Tsvetkova D, Mohamed A, Patel S, *et al*. Parafibromin, APC, and MIB-1 are useful markers for distinguishing parathyroid carcinomas from adenomas. Appl Immunohistochem Mol Morphol 2017;25:731-5.