Original article

Ultrastructure of Hyperfunctioning Parathyroid Glands: Does it Explain Various Patterns of ^{99m}Tc-sestamibi Uptake

Abdelhamid H. Elgazzar, Jehoran T. Anim[1], Shorouk F. Dannoon, Medhat M. Farghaly[2]

Departments of Nuclear Medicine, [1]Pathology and [2]Surgery, Faculty of Medicine, Kuwait University, Safat, Kuwait

Abstract

The aim of this study was to correlate the uptake of ^{99m}Tc-methoxy-isobutyl-isonitrile (MIBI) with ultra-structural features of parathyroid adenomas. Twenty patients with proven primary hyperparathyroidism were evaluated prospectively. Preoperative double-phase ^{99m}Tc-MIBI scintigraphy was performed in all patients and the degree of tracer uptake by the parathyroid lesions was assessed visually and semi-quantitatively. The excised glands were examined histologically and ultrastructurally, and their features were correlated with the degree of the radiotracer uptake. At surgery, 21 parathyroid adenomas were removed (double adenoma in one patient and a solitary adenoma in each of the remaining 19 patients). ^{99m}Tc-MIBI scan detected 18 of the 21 adenomas. There was positive correlation between the degree of ^{99m}Tc-MIBI uptake and the mitochondrial contents of the parathyroid adenoma cells. Four adenomas with intense uptake had high content of mitochondria in the cells. The three false-negative scans had low-to-moderate mitochondrial content. ^{99m}Tc-MIBI uptake is related to the mitochondrial content of the parathyroid adenoma cells.

Keywords: ^{99m}Tc- methoxy-isobutyl-isonitrile, electron microscopy, mitochondria, parathyroid adenoma, radionuclide

Introduction

Since a single parathyroid adenoma is an underlying abnormality in about 85% of cases of primary hyperparathyroidism,[1] there would be no need to explore both sides of the neck with potentially increased morbidity if a sensitive imaging modality could localize the abnormal gland preoperatively.[2,3] Although experienced neck surgeons can achieve nearly 95% success rate of parathyroidectomy after bilateral neck exploration without prior localizing study,[4,5] a preoperative localization study would decrease operative time and morbidity. The new trend is toward minimally invasive surgery which depends not only on surgeons' experience but also on a sensitive and accurate imaging technique.[6-9]

In the last few years, ^{99m}Tc-methoxy-isobutyl-isonitrile (MIBI) scan has become the most sensitive and cost-effective modality for preoperative localization of hyperfunctioning parathyroid tissue.[10-13] If a single parathyroid adenoma is detected, a unilateral scan-directed neck exploration can be performed. Due to a wide variation in scintigraphic techniques,[14,15] the reported sensitivities of ^{99m}Tc-MIBI scan range from 80% to 100%. Bénard *et al*. reported a large adenoma which was missed on ^{99m}Tc-MIBI scan seemingly due to a rapid washout along with the presence of

Address for correspondence:
Prof. Abdelhamid H. Elgazzar, Department of Nuclear Medicine, Faculty of Medicine, Kuwait University, PO Box 24923, Safat 13110, Kuwait.
E-mail: aelgazzar49@hotmail.com

Access this article online

Quick Response Code:

Website: www.wjnm.org

DOI: 10.4103/1450-1147.203073

How to cite this article: Elgazzar AH, Anim JT, Dannoon SF, Farghaly MM. Ultrastructure of hyperfunctioning parathyroid glands: Does it explain various patterns of ^{99m}Tc-sestamibi uptake. World J Nucl Med 2017;16:145-9.

few oxyphil cells.[16] The cause of this is not well established. Significant P-glycoprotein or multidrug resistance-related protein expression was reported to limit the sensitivity of ^{99m}Tc-MIBI imaging in localizing parathyroid adenomas.[17]

Exploring the pathophysiology of parathyroid gland might clarify why some small adenomas can accumulate ^{99m}Tc-MIBI while others, even large ones cannot. This information might help refine techniques to improve the accuracy of the ^{99m}Tc-M1BI parathyroid scan. Thallium-201 (^{201}Tl) along with ^{99m}Tc-pertechnetate ($^{99m}TcO_4^-$) were used for years to localize parathyroid lesions before ^{99m}Tc-MIBI essentially replaced them. One study which correlated ^{201}Tl scintigraphy with ultrastructural alterations in hyperfunctioning parathyroid lesions has suggested that the ability of this scan to localize the abnormal glands might depend on upon the amount of mitochondria-rich oxyphil cells.[18] In general, the uptake mechanisms of ^{201}Tl and ^{99m}Tc-MIBI differ significantly. ^{201}Tl uptake depends predominantly on Na^+ and K^+ ATPase pumps while ^{99m}Tc-MIBI uptake has been related to the mitochondria. Accordingly, the findings regarding the correlation of ^{201}Tl uptake by parathyroid adenomas and ultra-structural changes may not be applicable to ^{99m}Tc-MIBI. We conducted this study in an attempt to correlate the degree of MIBI uptake with the ultrastructural components of abnormal parathyroid glands.

Materials and Methods

Twenty patients (16 women and 4 men; mean age 44.6 years; range 13–66 years) with biochemically documented [Table 1] primary hyperparathyroidism underwent parathyroidectomy after ^{99m}Tc-MIBI parathyroid localization studies. The preoperative clinical work-up of each case included thyroid and liver function tests, mineral profile, renal profile, intact parathormone level, and for eight of the patients a 24-h urine calcium test was also performed. A portion of each surgical sample was examined by electron microscopy (EM) and the remaining portion by light microscopy (LM).

Table 1: Demographic and major biochemical data of patients

Criteria	Mean±SE	Median (range)
Age (years)	44.6±3.63	46 (13-66)
Corrected serum calcium*	2.95±0.08	2.82 (2.63-4.1)
Serum phosphate†	0.80±0.06	0.82 (0.26-1.26)
Intact-PTH‡	37.16±11.75	16.35 (9.8-192)
24-h urine calcium§	10.71±4.19	7.45 (2.38-38.6)

Normal ranges: *2.20-2.60 mmol/L; †0.80-1.40 mmol/L; ‡1.3-7.7 pmol/L; §1.2-10 mmol/day. SE: Standard error; PTH: Parathormone

Imaging methods

Anterior planar images of the neck and chest were acquired at 15 and 120 min after intravenous injection of 555 MBq (15 mCi) of ^{99m}Tc-MIBI and on 12 patients a 60 min postinjection scan was performed. A large-field-of-view of dual-head gamma camera equipped with a low energy, parallel-hole, and high-resolution collimator was used. A single-photon emission computed tomography (SPECT) study of the neck and mediastinum was performed using a 128 × 128 matrix and 1.33 zoom with an acquisition time of 25 s per frame for a total of 64 frames. For proper interpretation of the scan, an adjunctive $^{99m}TcO_4^-$ thyroid scan was done for five of these patients to delineate the thyroid and exclude thyroid pathology. A distinct increase of ^{99m}Tc-MIBI uptake relative to thyroid tissue seen on late images was considered positive for abnormal parathyroid tissue on planar images as well as on SPECT studies. Visual analysis was done on the planar delayed image and scaled as follows: 0-negative scan, 1-mildly increased uptake, 2-moderately increased uptake, 3-intense uptake. In addition, a semi-quantitative analysis was obtained by drawing region of interest (ROI) around the visualized lesion and a comparable one over the adjacent background. A count ratio of parathyroid to background (P/B) was calculated using the average count in each ROI. P/B ratio could be obtained only for 16 patients as computer data were lost for the remaining four patients. The scans were independently reviewed by two nuclear medicine physicians. The consultation of a third physician was used to resolve a disagreement about the positivity of one scan.

The surgical approach consisted of a horizontal cervicotomy and exploration of the four parathyroid glands. Glands with increased uptake on ^{99m}Tc-MIBI scan as well as grossly enlarged glands were removed.

Histopathological and electron microscopy methods

The surgical specimen was divided into two portions. The first portion was fixed in 3% glutaraldehyde in Millonig's phosphate buffer, followed by 1% osmium tetroxide solution in Millonig's buffer then loaded/embedded on epoxy resin. To be examined in a JEOL transmission electron microscope (JEM-1200 EX-TT, Japan), the ultra-microtome sections of the fixed surgical specimen were stained using uranyl acetate and lead citrate.

In general, the predominant cell in the section of each specimen was analyzed with a magnification power of 20,000 for most of the cases, and a semi-quantitative assessment of the ultra-structural features was based on visual observation. The cellular organelles of interest that were assessed included the mitochondrial content of cells, Golgi apparatus, endoplasmic reticulum, cisternae, lipid droplets, and residual bodies. The mitochondrial contents were semi-quantitatively assessed as follows: Mild (mitochondria were scattered), moderate (many mitochondria were found), and high (cell packed with mitochondria).

The remaining portion of the fresh specimen was fixed in 10% buffered formalin (pH 7.0) and processed routinely into paraffin. Five micrometers sections were stained with hematoxylin and eosin for histological examination to confirm the presence of parathyroid adenoma and for semi-quantitative assessment of proportions of different constituent cells.

Statistical methods

Statistical analyses were performed using IBM SPSS statistical software version 12.0. (SPSS for Windows, Chicago, SPSS Inc.). The Pearson's correlation coefficient was used to correlate both the degree of ^{99m}Tc-MIBI uptake as well as P/B ratio with the EM findings. $P < 0.05$ was considered as an indication of a statistically significant correlation.

Results

Twenty-one lesions were removed from the 20 patients and confirmed histopathologically to be adenomas. All lesions were orthotropic in the neck. The average weight of the adenomas was 2.35 g, ranging from 0.3 to 16.6 g. The P/B ratio of MIBI positive scans was in the range of 1.52–3.7, reflecting good visualization of the adenomas. Nine of the patients had lower left adenomas at surgery ranging in weight between 0.3 and 16.6 g averaging at 2.7 ± 5.3 g and patient #7 had negative MIBI scan. Ten patients had lower right adenomas ranging between 0.6 and 3.6 g averaging at 2.2 ± 1.2 g and patient #17 had negative MIBI scan. Patient #9 had 1.9 g left upper adenoma with parathyroid/background ratio of 2.64. Patient #18 had 1.3 g right upper adenoma, and the MIBI scan was negative. Preoperative ^{99m}Tc-MIBI scintigraphy correctly identified and localized 18 of the 21 adenomas. Three of the 20 scans were false-negatives. One scan (No. 14) demonstrated two true positive parathyroid adenomas, while each of the remaining 16 scans localized a single adenoma. Accordingly, the sensitivity was 86%, and specificity was 100%.

The results of LM and EM shows positive correlation between the visually determined degree of ^{99m}Tc-MIBI uptake in parathyroid adenomas and high amounts of mitochondria in the adenomas ($P = 0.006$), as well as positive correlation between P/B (parathyroid/background) ratios and corresponding high amounts of mitochondria in the adenomas ($P = 0.034$). In four adenomas with intense MIBI uptake, high amount of mitochondria was seen [Figure 1], and each of the three scan-negative adenomas showed the low-to-moderate amount of mitochondria. Other ultrastructural parameters such as Golgi apparatus, endoplasmic reticulum, residual bodies, or lipid droplets did not have any statistically significant correlations with ^{99m}Tc-MIBI uptake.

Both visual uptake and P/B ratio analyses significantly correlated with weight of the adenomas ($P = 0.011$, 0.007, respectively) [Table 2]. Two of the adenomas missed on ^{99m}Tc-MIBI scan were small (<1.0 g) whereas the third one was larger (1.3 g). Serum intact parathormone and calcium levels did not correlate with the degree of uptake.

In the cases with both planar and SPECT studies, SPECT was not superior to planar imaging in detecting or localizing the parathyroid adenomas, since all lesions were found at the expected anatomic locations as in planar studies. No additional adenomas were detected by SPECT. Thyroid scan done on five patients, including the patient with the smallest scintigraphically undetected adenoma, failed to improve the sensitivity or specificity of ^{99m}Tc-MIBI scan.

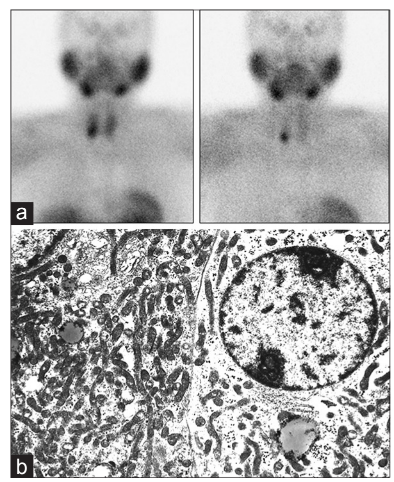

Figure 1: (a) Methoxy isobutyl isonitrile scan of a 41-year-old male showing left lower parathyroid adenoma. (b) Electron microscopy picture showing marked amount of mitochondria in an oxyphil cell

Table 2: Scintiography and electron microscopy results

Visual evaluation of scan	Number of patients	Average P/B* ratio from scan	Mitochondria expression via electron microscopy
0	3	0	2 out of 3 - normal
1	8	1.73±0.21	7 out of 8 - moderate
2	5	2.13±0.33	4 out of 5 - moderate
3	5	3.08±0.38	4 out of 5 - dense

*Parathyroid to background uptake ratio

Discussion

The vast majority of parathyroid adenomas are composed predominantly of tightly packed chief cells, often intermingled with oxyphil and clear cells. Oxyphil adenomas are uncommon. Closely packed mitochondria are characteristic of oxyphil cells while chief cells contain not only moderate to high mitochondrial content, but also prominent Golgi apparatus and endoplasmic reticulum. Water-clear cells are vacuolated with marked distension of organelles. Each of the three cell types may contain varying amounts of lipid droplets and residual bodies. No clinical studies have been reported to correlate the degree of ^{99m}Tc-MIBI uptake seen on preoperative imaging studies in hyperfunctioning parathyroid glands with the ultrastructural changes of these glands.

Our study showed a positive correlation between the degree of ^{99m}Tc-MIBI uptake and high content of mitochondria in the parathyroid adenomas. Since the oxyphil cell is the type found to be rich in mitochondria, our findings were similar to the results of an LM study conducted by Carpentier *et al*.[18] which correlated ^{99m}Tc-MIBI uptake on delayed images with oxyphil cells in parathyroid adenomas. Other studies did not find a correlation between the degree of hypercellularity of oxyphil cell and ^{99m}Tc-MIBI uptake,[19] or a relation between the percentage of chief and oxyphil cells and scintigraphic findings.[20]

These results are in agreement with a recent *in vitro* study in which parathyroid tissues removed from patients with hyperparathyroidism were incubated with ^{99m}Tc-MIBI followed by enzymatic isolation of mitochondria (confirmed by EM). From this work, the mitochondria were found to be the principal organelles of ^{99m}Tc-MIBI uptake by parathyroids.[21]

Two of the three missed adenomas weighed <1 g and the third one was 1.3 g their EM examination revealed no abundance of mitochondria. The adenoma in No. 12 had 35% oxyphil cells that was faintly visualized by ^{99m}Tc-MIBI scan probably because the mitochondria in oxyphil cells as was later shown by EM examination were scattered.

Conclusion

The major limitation of our study was the small number of glands examined, especially the ones that were not detected by ^{99m}Tc-MIBI. However, our data suggest that, in cases of primary hyperparathyroidism, the basis of ^{99m}Tc-MIBI scintigraphy in the detection of hyperfunctioning parathyroid largely depends on upon the presence of abundant amounts of mitochondria within the hyperfunctioning parathyroid adenomas.

Financial support and sponsorship

Kuwait University Research Grant No. MNO1/01.

Conflicts of interest

There are no conflicts of interest.

References

1. Wang C. The anatomic basis of parathyroid surgery. Ann Surg 1976;183:271-5.
2. Alenezi SA, Asa'ad SM, Elgazzar AH. Scintigraphic parathyroid imaging: Concepts and new developments. Res Rep Nucl Med 2015;5:9-18.
3. Elgazzar AH, Alenezi SA. Parathyroid gland. In: Elgazzar AH, editor. Pathophysiologic Basis of Nuclear Medicine. 3rd ed. Berlin: Springer; 2014. p. 9-18.
4. Shaha AR, Jaffe BM. Cervical exploration for primary hyperparathyroidism. J Surg Oncol 1993;52:14-7.
5. Mitchell BK, Merrell RC, Kinder BK. Localization studies in patients with hyperparathyroidism. Surg Clin North Am 1995;75:483-98.
6. Denham DW, Norman J. Cost-effectiveness of preoperative sestamibi scan for primary hyperparathyroidism is dependent solely upon the surgeon's choice of operative procedure. J Am Coll Surg 1998;186:293-305.
7. O'Doherty MJ, Kettle AG. Parathyroid imaging: Preoperative localization. Nucl Med Commun 2003;24:125-31.
8. Hindié E, Ugur O, Fuster D, O'Doherty M, Grassetto G, Ureña P, *et al.* 2009 EANM parathyroid guidelines. Eur J Nucl Med Mol Imaging 2009;36:1201-16.
9. Kim YI, Jung YH, Hwang KT, Lee HY. Efficacy of 99mTc-sestamibi SPECT/CT for minimally invasive parathyroidectomy: Comparative study with 99mTc-sestamibi scintigraphy, SPECT, US and CT. Ann Nucl Med 2012;26:804-10.
10. Mihai R, Simon D, Hellman P. Imaging for primary hyperparathyroidism – An evidence-based analysis. Langenbecks Arch Surg 2009;394:765-84.
11. Patel CN, Salahudeen HM, Lansdown M, Scarsbrook AF. Clinical utility of ultrasound and 99mTc sestamibi SPECT/CT for preoperative localization of parathyroid adenoma in patients with primary hyperparathyroidism. Clin Radiol 2010;65:278-87.
12. Bleier BS, LiVolsi VA, Chalian AA, Gimotty PA, Botbyl JD, Weber RS. Technetium Tc 99m sestamibi sensitivity in oxyphil cell-dominant parathyroid adenomas. Arch Otolaryngol Head Neck Surg 2006;132:779-82.
13. McBiles M, Lambert AT, Cote MG, Kim SY. Sestamibi parathyroid imaging. Semin Nucl Med 1995;25:221-34.
14. Perez-Monte JE, Brown ML, Shah AN, Ranger NT, Watson CG, Carty SE, *et al.* Parathyroid adenomas: Accurate detection and localization with Tc-99m sestamibi SPECT. Radiology 1996;201:85-91.
15. Hindié E, Mellière D, Perlemuter L, Jeanguillaume C, Galle P. Primary hyperparathyroidism: Higher success rate of first surgery after preoperative Tc-99m sestamibi-I-123 subtraction scanning. Radiology 1997;204:221-8.
16. Bénard F, Lefebvre B, Beuvon F, Langlois MF, Bisson G. Rapid washout of technetium-99m-MIBI from a large parathyroid adenoma. J Nucl Med 1995;36:241-3.
17. Kao A, Shiau YC, Tsai SC, Wang JJ, Ho ST. Technetium-99m methoxyisobutylisonitrile imaging for parathyroid adenoma: Relationship to P-glycoprotein or multidrug resistance-related protein expression. Eur J Nucl Med Mol Imaging 2002;29:1012-5.

18. Carpentier A, Jeannotte S, Verreault J, Lefebvre B, Bisson G, Mongeau CJ, *et al.* Preoperative localization of parathyroid lesions in hyperparathyroidism: Relationship between technetium-99m-MIBI uptake and oxyphil cell content. J Nucl Med 1998;39:1441-4.
19. Ishibashi M, Nishida H, Okuda S, Suekane S, Hayabuchi N. Localization of parathyroid glands in hemodialysis patients using Tc-99m sestamibi imaging. Nephron 1998;78:48-53.
20. Piñero A, Rodriguez JM, Ortiz S, Soria T, Bermejo J, Claver MA, *et al.* Relation of biochemical, cytologic, and morphologic parameters to the result of gammagraphy with technetium 99m sestamibi in primary hyperparathyroidism. Otolaryngol Head Neck Surg 2000;122:851-5.
21. Hetrakul N, Civelek AC, Stagg CA, Udelsman R. *In vitro* accumulation of technetium-99m-sestamibi in human parathyroid mitochondria. Surgery 2001;130:1011-8.