ORIGINAL ARTICLE
J Korean Thyroid Assoc
Vol. 3, No. 1, May 2010

Thyroid Papillary Carcinomas with Extensive Calcification and Bone Metaplasia: A Clinicopathological Analysis of 41 Carcinoma Cases and Comparison with 30 Benign Cases with Extensive Calcification and Bone Metaplasia

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Background and Objectives: The aim of the present study was to investigate the clinicopathological characteristics and implications of papillary carcinoma with extensive calcification and bone metaplasia (PTCECB), and to determine the differences between PTCECB and benign nodules with calcification and bone metaplasia (BNECB). **Materials and Methods:** We retrospectively reviewed the slides of 41 PTCECB cases and 30 BNECB cases. **Results:** Only four (9.8%) cases of PTCECB demonstrated psammoma bodies. Twenty eight (68.3%) cases of PTCECB showed prominent follicular architectures with occasional bland-appearing thyrocytes showing diffuse immunohistochemical expression for CK19 and galectin-3. All BNECBs revealed expanding tumor margins and were intrathyroidally located. In contrast, PTCECB showed expanding tumor margins in 26 (63.4%) cases (p=0.001) and extrathyroidal extension in 20 (48.8%) cases (p=0.000). **Conclusion:** PTCECB showed characteristics of prominent follicular architecture and an absence of psammoma bodies and should be differentiated from BNECB by histological features such as extrathyroidal extension and characteristic nuclear features of papillary thyroid carcinoma combined with immunohistochemical study such as CK19 and galectin-3.

Key Words: Calcification, Ossification, Papillary carcinoma, Thyroid gland

Introduction

Calcification in thyroid glands is a common finding in the practice of surgical pathology. Although calcification is noted in both benign and malignant thyroid disease,[1-4] calcification is most frequently associated with thyroid cancer such as papillary carcinoma.[5-8] Still, the exact clinical significance of calcification is not known. Calcification occasionally gives rise to bone metaplasia and mature bone-containing marrow tissue.[9,10] It is easy for pathologists to neglect calcification and bone metaplasia because they are very common findings. Calcification and bone metaplasia in benign thyroid disease occur most frequently in multinodular hyperplasia,[2-4] and the findings are more common with increasing goiter duration. Among malignant thyroid tumors, papillary thyroid carcinoma (PTC) most frequently demonstrates calcification and bone metaplasia.[2,5,11]

Although the clinical and pathological significances of calcification in papillary carcinoma are not well

Received November 18, 2009 / Accepted January 25, 2010
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known, previous literature has reported that psammoma bodies were associated with gross lymph node metastasis, high tumor stage, and decreased disease-free survival, and that stromal calcification showed associations with old age, gross lymph node metastasis, and high tumor stage.[12] Finally, bone formation is known to be associated with old age.[12] However, to our knowledge, a study on the clinical and pathological significances of papillary carcinoma with extensive calcification and bone metaplasia (PTCECB) has not been performed.

The purpose of this study was to investigate the clinicopathological characteristics and implications of PTCECB and to determine the differences between PTCECB and benign thyroid nodules with extensive calcification and bone metaplasia (BNECB).

Materials and Methods

Patient selection

We undertook a retrospective review of all patients diagnosed with PTC with bone metaplasia or ossification who underwent surgery at Severance Hospital from January 1995 to December 2008. Among 3,796 patients who underwent thyroidectomies for PTC, 41 cases with PTCECB were included. The study was approved by the Institutional Review Board of Yonsei University Severance Hospital. Clinical data was obtained from patients' medical records and included age at diagnosis and sex. As a control group, 30 BNECB cases were included.

Pathological evaluation

Thyroid glands with extensive calcification and bone metaplasia were decalcified for more than 24 hours using a rapid decalcifying solution (Calci-Clear Rapid, National Diagnostics, Atlanta, GA, USA). Before decalcification, thyroid glands were fixed in a 10% formaldehyde solution for 24 hours. All grossly-identified tumors were embedded in tissue blocks. Formalin-fixed, paraffin-embedded tissue blocks were used. H&E-stained sections were reviewed by two pathologists (JS Koo and SW Hong). Pathological parameters of papillary carcinomas such as size, tumor margin, histological subtype, presence of psammoma bodies, stromal calcification, lymph node involvement, extrathyroidal extension, and combined lymphocytic thyroiditis were included. In addition, pathological features of bone formation such as the proportion of stromal calcification and metaplastic bone among tumors, metaplastic bone location [localized (central or peripheral zone) or diffuse (central and peripheral zone)], and combined components (fat and/or hematopoietic cells) were included. These variables were also investigated in the control group. Psammoma bodies, stromal calcification, and bone formation were defined according to the previous literature.[12] In the cases of papillary carcinoma with stromal calcification and bone formation, only cases with more than 50% total tumor area of stromal calcification and bone formation (defined as extensive bone formation in this study) were included.

Statistical analysis

Data was statistically processed using SPSS for Windows, version 12.0 (SPSS, Chicago, IL, USA). Student's t and Fisher's exact tests were used for analyzing continuous and categorical variables, respectively, to determine statistical significances among clinicopathological parameters between the study and control groups. Statistical significance was defined as $p<0.05$.

Results

Clinicopathological features of PTCECB

The mean patient age was 55.3±11.6 years. There were 36 females (87.8%) and five males (12.2%) who had a mean tumor size of 1.5±0.5 cm. PTCECB was located in the right lobe in 24 (58.5%) cases and the left lobe in 17 (41.5%) cases. Bilateral thyroid gland involvement by PTC was noted in 19 (46.3%) cases. At low magnification, 26 (63.4%) cases showed expanding margins with clear distinctions from the surrounding normal thyroid tissue (Fig. 1A). Infiltrative growth patterns were noted in 15 (36.6%) cases. Some cases showed expanding margins that demonstrated fibrous capsules around the tumors.

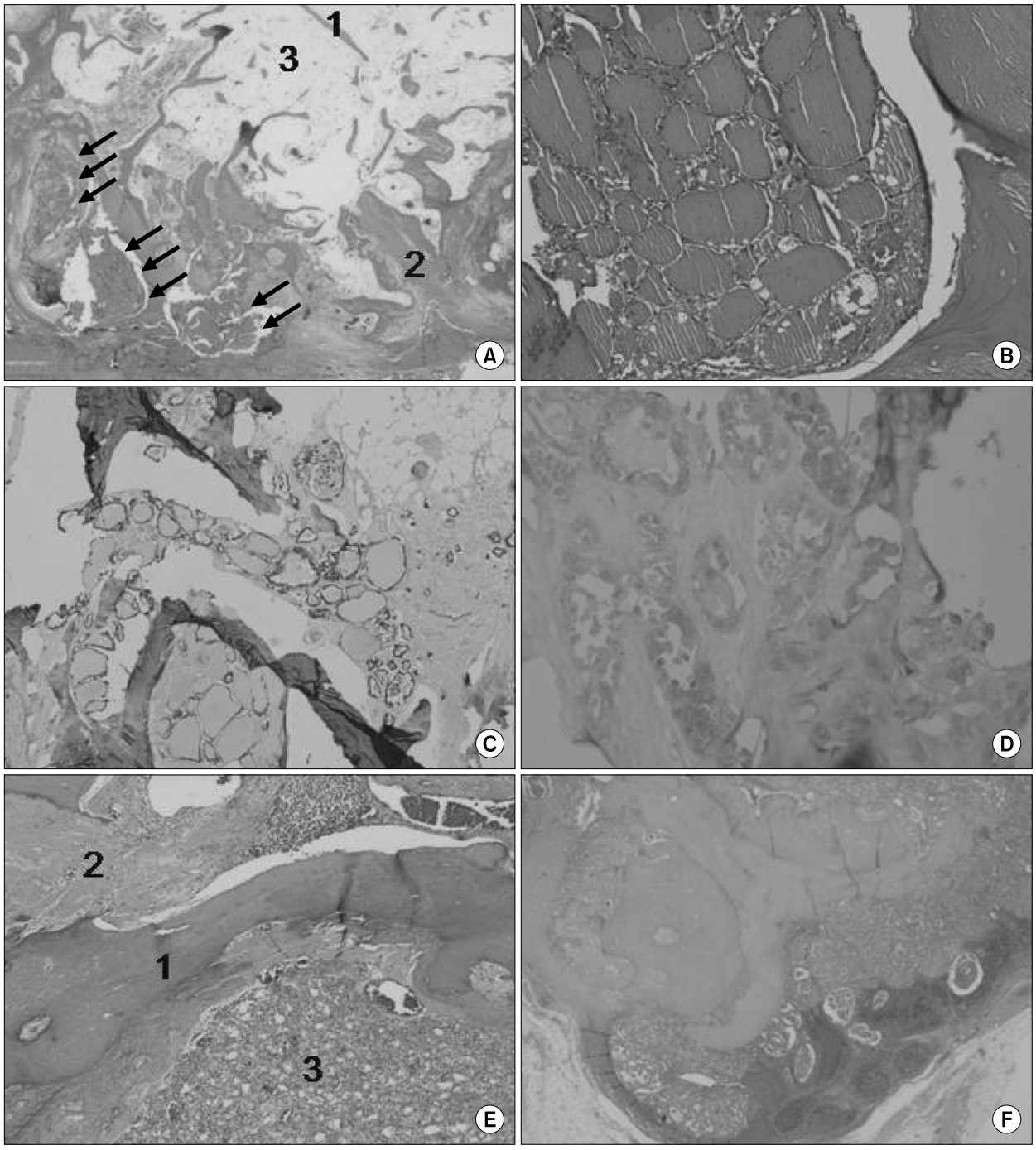

Fig. 1. Histological features of PTCECB. In a low magnification field, a well-circumscribed nodule composed of mature bone trabeculae (1), stromal calcification (2), and adipose tissue (3) is noted. (A) Thyroid cancer tissue is identified in the small and peripheral area of the tumor (arrow). (B) In a higher magnification field, this thyroid cancer tissue is mostly made of bland-looking thyroid follicle. Immunohistochemical tests for (C) CK19 and (D) galectin-3 revealed diffuse expression in bland-looking thyroid follicle, therefore PTC can be diagnosed. (E) The transition from stromal calcification to ossification (1) is noted and between bone trabeculae dense sclerotic fibrosis (2) and thyroid follicular tissue (3) are observed. (F) Metastatic PTC shows extensive calcification and bone metaplasia in the lymph node (A: H&E, ×12, B: H&E, ×200, C: CK19, ×200, D: galectin-3, ×200, E: H&E, ×12, F: H&E, ×12).

The histological diagnosis was papillary carcinoma with more papillary architecture than follicular architecture (PTCp) in 11 (26.8%) cases, papillary carcinoma with more follicular architecture than papillary

architecture (PTCf) in 15 (26.8%) cases, papillary carcinoma, follicular variant (PTCF) in 13 (31.7%) cases, and diffuse sclerosing variant papillary carcinoma (DSVPC) in two (4.9%) cases. Although PTCECB showed papillary and/or follicular architecture with various proportions, cases showing more follicular than papillary architecture were more common. The observed papillary architectures showed large and sclerotic papillary cores and four cases demonstrated cystic degeneration. Follicular architecture containing prominent colloids was composed of nuclei showing characteristics of papillary carcinoma such as nuclear clearing, grooves, and inclusion. However, in some cases, most follicles consisted of bland-looking thyrocytes (Fig. 1B), which made it difficult to diagnose papillary carcinoma. Therefore, in these cases, extensive tissue sampling was performed to detect typical nuclear features of PTC such as nuclear clearing, groove, and pseudoinclusion. In addition, immunohistochemistry using CK19 (Fig. 1C) and galectin-3 (Fig. 1D) was helpful for the final diagnoses in these cases. Extrathyroidal extension was noted in 20 (48.8%) cases. Psammoma bodies were observed in 4 (9.8%) cases and stromal calcification was noted in 40 (97.6%) cases. This stromal calcification was accompanied by dense stromal fibrosis and sclerosis, and occasionally it was noted that the ossification process began with stromal calcification (Fig. 1E). The proportion of bone formation among the total tumor area was 60.4±11.6% (mean±SD). The appearance of metaplastic bone was mature bony trabeculae where an osteoblastic rim was noted. In 23 (56.1%) cases, marrow fat tissue was identified between mature bony trabeculae, and occasionally hematopoietic cells were observed in marrow fat tissue. Sclerotic and fibrotic tissues were noted between mature bony trabeculae except in marrow fat tissue, and infarcted thyroid tissue was observed on occasion. The bone formation zone was localized in sixteen (39.0%) cases, and the diffuse in 25 (61.0%) cases. Lymphocytic thyroiditis was indentified in 13 (31.7%) cases and lymph node metastasis was identified in 26 (63.4%) cases. Of these, two cases demonstrated extensive bone formation in metastatic tumors (Fig. 1F). Tumor recurrence was noted in 2 (4.9%) cases, and there was no patient's death due to PTC.

Table 1 illustrates the comparison of the clinicopathological characteristics among the histological subtypes of PTCECB. Histological subtypes of PTCECB are divided into PTC, follicular variant and PTC, non-follicular variant. Most parameter did not show

Table 1. Comparison of the clinicopathological characteristics among the histological subtypes of papillary carcinoma with extensive bone metaplasia and mature bone formation

Parameters	Histological subtype		p-value
	PTC, follicular variant n=13 (%)	PTC, non-follicular variant* n=28 (%)	
Age (yr, mean±SD)	58.9±11.6	53.7±11.5	0.187
Sex			0.672
Female	11 (84.6)	25 (89.3)	
Male	2 (15.4)	3 (10.7)	
Tumor size (cm, mean±SD)	1.3±0.5	1.6±0.5	0.160
Tumor site			0.678
Right	7 (53.8)	17 (60.7)	
Left	6 (46.2)	11 (39.3)	
Bilateral involvement	7 (53.8)	12 (42.9)	0.511
Tumor margin			0.598
Expanding	9 (69.2)	17 (60.7)	
Infiltrative	4 (30.8)	11 (39.3)	
Tumor extension			0.368
Intrathyroidal	8 (61.5)	13 (46.4)	
Extrathyroidal	5 (38.4)	15 (53.6)	
Psammoma bodies	1 (7.7)	3 (10.7)	0.762
Stromal calcification	13 (100.0)	27 (96.4)	0.490
Proportion of bone and stromal calcification (%, mean±SD)	63.0±13.7	59.3±10.5	0.337
Fatty marrow tissue	7 (53.8)	16 (57.1)	0.843
Bone formation zone			0.460
Localized	4 (30.8)	12 (42.9)	
Diffuse	9 (69.2)	16 (57.1)	
Lymphocytic thyroiditis	1 (7.7)	12 (42.9)	0.024
Lymph node involvement	8 (61.5)	18 (64.3)	0.911
Cystic change		6 (21.4)	0.071
Tumor recurrence	1 (7.7)	1 (3.6)	0.569

*includes PTCf (15 cases), PTCp (11 cases), and DSVPC (2 cases). PTC: papillary thyroid carcinoma, PTCf: papillary carcinoma with more follicular architecture than papillary architecture, PTCp: papillary carcinoma with more papillary architecture than follicular architecture, DSVPC: diffuse sclerosing variant papillary carcinoma

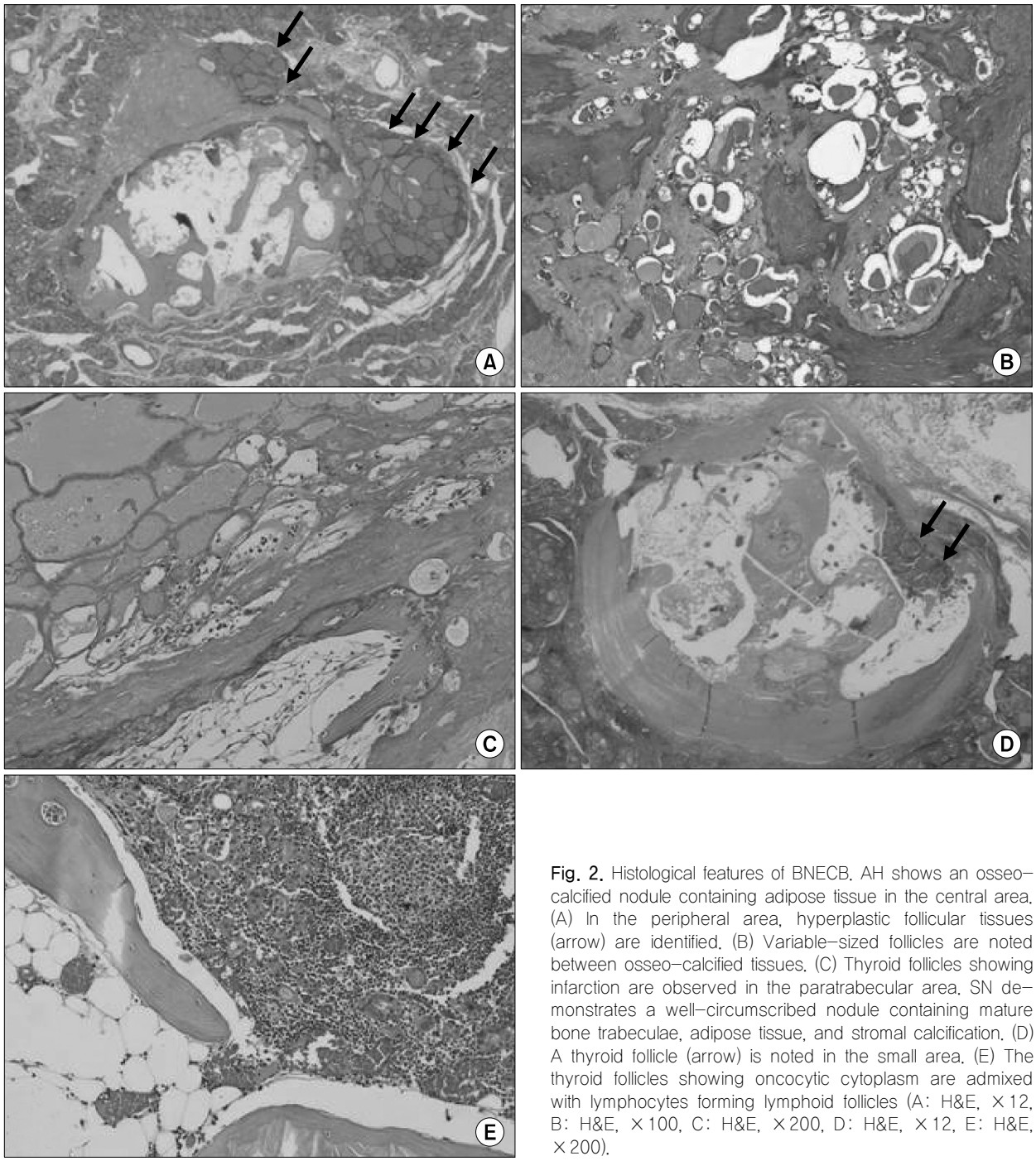

Fig. 2. Histological features of BNECB. AH shows an osseo-calcified nodule containing adipose tissue in the central area. (A) In the peripheral area, hyperplastic follicular tissues (arrow) are identified. (B) Variable-sized follicles are noted between osseo-calcified tissues. (C) Thyroid follicles showing infarction are observed in the paratrabecular area. SN demonstrates a well-circumscribed nodule containing mature bone trabeculae, adipose tissue, and stromal calcification. (D) A thyroid follicle (arrow) is noted in the small area. (E) The thyroid follicles showing oncocytic cytoplasm are admixed with lymphocytes forming lymphoid follicles (A: H&E, ×12, B: H&E, ×100, C: H&E, ×200, D: H&E, ×12, E: H&E, ×200).

statistical significance between PTC, follicular variant and PTC, non-follicular variant. Lymphocytic thyroiditis was more frequently observed in PTC, non-follicular variant (p=0.024). Cyctic change of the tumor was noted only in PTC, non-follicular variant.

Clinicopathological features of BNECB

The mean age of the patients was 55.7±10.3 years (mean±SD). Only one (3.3%) patient was male and the mean tumor size of all patients was 1.3±0.7 cm (mean±SD). BNECB were located in the right lobe in 17 (56.7%) cases and the left lobe in 13 (43.3%) cases. All cases showed expanding margins and were intrathyroidally located. Histological diagnosis was adenomatous hyperplasia (AH) in 11 (36.7%) cases and single calcified nodule (SN) in 19 (63.3%)

cases. Some cases of adenomatous hyperplasia showed multiple adenomatoid nodules where bone metaplasia had occurred (Fig. 2A). Thyroid tissue between mature bone trabeculae showed variable-sized hyperplastic follicles (Fig. 2B) and infarcted appearances (Fig. 2C). Thyroid follicles consisted of bland-looking nuclei and prominent colloid materials. A single calcified nodule composed of dense fibrosis, stromal calcification, and metaplastic bone tissue (Fig. 2D) was observed during the course of this study. Thyroid follicles were rarely identified and identifiable follicles had atrophic and degenerative appearances (Fig. 2E). No tumors demonstrated psammoma bodies and 29 (96.7%) cases showed stromal calcification. The proportion of bone metaplasia among the tumor areas was 71.0±17.8% (mean±SD). Fatty marrow tissue was identified in 15 (50.0%) cases. The bone formation area was localized in 9 (30.0%) cases, and diffuse in 21 (70.0%) cases. Lymphocytic thyroiditis was present in 8 (26.7%) cases. Eleven (36.6%) cases showed PTC on the contralateral side and one (3.3%) case showed anaplastic carcinoma on the contralateral side.

Table 2 shows a clinicopathological characteristics comparison between AH and SN with extensive bone metaplasia and mature bone formation. The mean tumor size of AH was 2.0±0.8 cm, which was larger than that of SN (0.9±0.3 cm, p=0.000). The proportion of bone metaplasia among the tumor area in SN was 78.9±16.9% (mean±SD), which was higher than that of AH (52.2±9.0%, p=0.001). Eight (42.1%) cases of SN were associated with lymphocytic thyroiditis, but AH was not associated with lymphocytic thyroiditis (p=0.012). Eleven (57.9%) cases of SN showed malignant thyroid cancer (ten PTC, one AC) in the contralateral thyroid gland, but only 1 (9.1%) case of AH demonstrated PTC in the contralateral thyroid gland (p=0.009).

Table 2. Comparison of the clinicopathological characteristics between the histological subtypes of benign thyroid nodules with extensive bone metaplasia and mature bone formation

Parameters	Histological diagnosis		p-value
	Adenomatous hyperplasia n=11 (%)	Single calcified nodule n=19 (%)	
Age (yr, mean±SD)	55.2±9.6	55.9±11.0	0.867
Sex			0.367
Female	10 (90.9)	19 (100.0)	
Male	1 (9.1)		
Tumor size (cm, mean±SD)	2.0±0.8	0.9±0.3	0.000
Tumor site			0.579
Right	6 (54.5)	11 (57.9)	
Left	5 (45.5)	8 (42.1)	
Stromal calcification	11 (100.0)	18 (94.7)	0.633
Proportion of bone and stromal calcification (%, mean±SD)	52.2±9.0	78.9±16.9	0.001
Fatty marrow tissue	4 (36.3)	11 (57.9)	0.256
Bone formation zone			0.282
Localized	2 (18.1)	7 (36.8)	
Diffuse	9 (81.9)	12 (63.2)	
Lymphocytic thyroiditis		8 (42.1)	0.012
Associated tumor			0.009
Contralateral PTC or AC	1 (9.1)	11 (57.9)	

PTC: papillary thyroid carcinoma, AC: anaplastic carcinoma

The comparison of clinicopathological characteristics between PTCECB and BNECB

Table 3 illustrates the comparisons of the clinicopathological characteristics between PTCECB and BNECB. Every case of BNECB revealed expanding tumor margins, while PTCECB showed expanding tumor margins in 26 (63.4%) cases (p=0.000). Every BNECB case was intrathyroidally located, but PTCECB revealed extrathyroidal extension in 20 (48.8%) cases (p=0.000). None of the BNECB cases revealed psammoma bodies while 4 (9.8%) PTCECB cases showed psammoma bodies (p=0.078). The proportion of bone metaplasia in BNECB was 71.0±17.8% (mean±SD), which was higher than that of PTCECB (60.4±11.6%, p=0.004). Clinicopathological parameters including age, sex, tumor size, tumor site, bone formation zone, incidence of stromal calcification, fatty marrow tissue, and lymphocytic thyroiditis were not significantly different between PTCECB and BNECB.

Discussion

PTC can demonstrate not only characteristics of

Table 3. Comparison of the clinicopathological characteristics between papillary carcinoma and benign thyroid nodules showing extensive bone metaplasia and mature bone formation

Parameters	Histological diagnosis		p-value
	PTC n=41 (%)	Benign nodule n=30 (%)	
Age (years, mean±SD)	55.3±11.6	55.7±10.3	0.901
Sex			0.185
Female	36 (87.8)	29 (96.7)	
Male	5 (12.2)	1 (3.3)	
Tumor size (cm, mean±SD)	1.5±0.5	1.3±0.7	0.339
Tumor site			0.875
Right	24 (58.5)	17 (56.7)	
Left	17 (41.5)	13 (43.3)	
Bilateral involvement	19 (46.3)		
Histological diagnosis			
PTC, papillary>follicular	11 (26.8)		
PTC, follicular>papillary	15 (36.6)		
PTC, follicular variant	13 (31.7)		
DSVPC	2 (4.9)		
Histological diagnosis			
Adenomatous hyperplasia		11 (36.7)	
Single calcified nodule		19 (63.3)	
Tumor margin			0.000
Expanding	26 (63.4)	30 (100.0)	
Infiltrative	15 (36.6)		
Tumor extension			0.000
Intrathyroidal	21 (51.2)	30 (100.0)	
Extrathyroidal	20 (48.8)		
Psammoma bodies	4 (9.8)	0 (0.0)	0.078
Stromal calcification	40 (97.6)	29 (96.7)	0.822
Proportion of bone and stromal calcification (%, mean±SD)	60.4±11.6	71.0±17.8	0.004
Fatty marrow tissue	23 (56.1)	15 (50.0)	0.611
Bone formation zone			0.432
Localized	16 (39.0)	9 (30.0)	
Diffuse	25 (61.0)	21 (70.0)	
Lymphocytic thyroiditis	13 (31.7)	8 (26.7)	0.646
Lymph node involvement	26 (63.4)		
Cystic change	6 (14.6)		
Associated lesion			
Contralateral PTC		11 (36.6)	
Contralateral AC		1 (3.3)	

PTC: papillary thyroid carcinoma, DSVPC: diffuse sclerosing variant papillary carcinoma, AC: anaplastic carcinoma

psammoma bodies, but also calcification or/and bone metaplasia. However, calcification and bone metaplasia also occur in benign thyroid disease.[1,5,13] The purpose of this study was to investigate the clinicopathological characteristics of PTCECB and to determine the differences between PTCECB and BNECB.

The age of patients with PTCECB was 55.3±11.6 years (mean±SD), which was higher than the mean age of patients with PTC (46 years).[14] Fifteen patients were older than 60 years (36.6%). These findings were similar to the results of a previous report in which PTC with stromal calcification and bone metaplasia occurred with more advanced age (>60 years) than did normal PTC.[12] In this study, one of the characteristics of PTCECB was prominence of the follicular architecture. PTCf (36.6%) and PTCF (31.7%) composed 68.3% of the study group. In addition, it was interesting that psammoma bodies were noted in only 4 (9.8%) cases, which consisted of two cases of DSVPC, known to show numerous psammoma bodies, and two cases of non-DSVPC. However, stromal calcification was observed in all cases except one. Therefore, it was suggested that psammoma bodies are not associated with stromal calcification and bone metaplasia. Psammoma bodies are microscopically concentric lamellated calcified structures, and were once thought to be formed by dystrophic calcification from the necrosis of intravascular tumor emboli.[15] Currently, psammoma bodies are thought to be formed by intracellular calcification of viable cells, an active biological process that fosters the degeneration and death of tumor cells and plays a barrier role in tumor growth.[16,17] Stromal calcification is formed by deposition of calcium phosphate in fibrotic stroma. Mature bone tissue is thought to be formed by osseous metaplasia from preexisting calcification, an assertion supported by findings that stromal calcification and metaplastic bone tissue co-exist, with a direct transition from calcification to ossification having been noted in several cases. Extensive stromal calcification and bone metaplasia were originally thought to result from dystrophic calcification of dying tumor cells. The old age of the patients, the dense fibrosis, and the occasionally-identified infarcted tumor tissue support this contention. However, calcification and ossification from tumor cell necrosis generally occur in

rapidly growing and large tumors, while the mean size of PTCECB in this study was 1.5 cm and PTC was a slow growing, representative tumor. Secondly, tumor cells give rise to calcification and ossification. Numerous cases in this study showed that stromal calcification and/or metaplastic bone tissue were in direct contact with viable tumor cells and that viable tumor follicles were located between mature bony trabeculae. In addition, this finding was supported by a previous study that reported that thyroid papillary carcinoma cell line BHP 18～21 and human thyroid papillary carcinoma tissue showed high levels of osteocalcin and Cbfa-1, the main transcription factor of osteocalcin, which suggested that thyrocytes share characteristics with osteoblasts.[18)]

PTCECB should be differentiated from BNECB. In this study, BNECB showed expanding margins (p=0.001), intrathyroidal location (p=0.000) in all cases, and higher proportions of stromal calcification and bone metaplasia (p=0.004) than PTCECB. However, a differential diagnosis cannot be constructed solely on these findings. The most important component in a differential diagnosis for a tumor is its cytologic features. Of PTCECB, 68.3% showed prominent follicular architecture and large areas that consisted of bland-looking thyrocytes that made differential diagnoses more difficult. In addition, because decalcification should be performed due to extensive calcification and bone metaplasia, a detailed evaluation of nuclear features can be difficult. Therefore, performing immunohistochemistry studies, such as those with CK19 or galectin-3, can be helpful when a differential diagnosis cannot be determined by histological features alone. PTC showed diffuse expressions for CK19 and galectin-3, but benign thyroid nodules with extensive calcification and bone metaplasia showed no expression or focal weak expression.[19-22)] However, antigen preservation for immunohistochemistry can be reduced due to the decalcification process. Decalcification solutions such as EDTA[23,24)] or mercuric chloride-formaldehyde fixative solution in 10% acetic acid[25)] did not affect the immunohistochemical results, while 10% formic acid or 5% nitric acid caused decreased antigenicity.[23,24)] In our experience, immunohistochemistry for CK19 and galectin-3 after decalcification did not cause significant diagnostic problems.

When BNECB was classified into AH and SN, SN demonstrated smaller tumor sizes (p=0.000), more proportional stromal calcification and bone metaplasia (p=0.001), and higher incidences of lymphocytic thyroiditis (p=0.012) and thyroid cancer in the contralateral side (57.9%, p=0.009) compared to AH. Therefore, SN demonstrated different characteristic features than AH, and we surmise that SN might represent nearly regressed PTC considering that thyroid cancer frequently co-existed in the opposite side. SN mainly consisted of dense fibrosis, stromal calcifications, and bone metaplasia, though viable thyroid follicle cells could not be identified in several cases. The pathological features of previously reported regressed PTC included dense hyalinized fibrous tissue without tumor cells, which was similar to the SN in this study.[26)] In addition, when a solitary calcified nodule was found, the possibility of malignancy was reported to be very high, which supported our suggestion.[11)]

In conclusion, PTCECB showed characteristics of prominent follicular architecture and the absence of psammoma bodies and should be differentiated from BNECB by histological features, such as extrathyroidal extension, and typical nuclear features of PTC which may be noted in focal tumor area, combined with immunohistochemistry studies such as those using CK19 and galectin-3.

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