World J Surg (2013) 37:2330–2335
DOI 10.1007/s00268-013-2107-5

World Journal of Surgery

The Prognostic Relevance of Psammoma Bodies and Ultrasonographic Intratumoral Calcifications in Papillary Thyroid Carcinoma

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Published online: 29 May 2013

Abstract

Background Although psammoma bodies (PB) are found in up to 50 % of papillary thyroid carcinomas (PTC), their clinicopathological significance remains uncertain. The aim of the present study was to determine the clinicopathological significance of PB and the correlation between PB and ultrasonographic intratumoral calcification in PTC.

Methods The clinicopathological parameters, ultrasonographic calcifications, and the presence of PB were evaluated in 258 surgically resected conventional PTC.

Results Psammoma bodies were found in 141 of 258 PTC (54.7 %). The presence of PB was significantly correlated with tumor multifocality, extrathyroidal extension, and lymph node metastasis ($P = 0.009$, $P = 0.004$, and $P < 0.001$, respectively), but not with the $BRAF^{V600E}$ mutation. Higher incidences of both intratumoral and extratumoral PB were found in overt PTC (>1 cm) than in papillary microcarcinomas (≤1 cm) ($P < 0.001$ and $P = 0.015$, respectively). Extratumoral PB were only identified in 48.9 % of 141 PTC with PB, and PTC with extratumoral PB showed higher incidences of tumor multifocality, extrathyroidal extension, and nodal metastasis compared to PTC with intratumoral PB ($P = 0.014$, $P = 0.005$ and $P = 0.001$, respectively). Ultrasonographic intratumoral calcification corresponded to clusters of intratumoral PB ($P < 0.001$) and was associated with nodal metastasis ($P = 0.026$).

Conclusions The findings of the present study suggest that the presence of PB may be a useful prognostic indicator of aggressive PTC behaviors. In addition, confirmation of ultrasonographic intratumoral calcification would be a useful decision-making criterion when determining the need for preoperative or intraoperative surveillance of nodal metastasis.

Introduction

Papillary thyroid carcinoma (PTC) is the most common type of thyroid cancer, accounting for 80–90 % of thyroid cancers[1–3]. The rising incidence of thyroid cancer is almost entirely attributable to the increased incidence of PTC [2–4]. Although most PTC have genetic alterations such as B-type Raf kinase (BRAF) mutations, RET/PTC translocations, and Ras mutations, the precise pathogenesis of PTC is unknown [5]. Clinicopathological characteristics such as patient age and gender, tumor size, extrathyroidal extension, and lymph node metastasis are useful prognostic factors in PTC [6].

Psammoma bodies (PB) are defined as spherical calcified foci with concentric laminations and are found in non-neoplastic conditions and neoplasms of the thyroid [6–8]. Psammoma bodies in PTC may be formed by necrosis and

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calcification of intravascular or intralymphatic tumor thrombi and by intracellular calcifications in the viable cells of the nidus [6]. The presence of PB is easily detected in cytologic or histologic specimens [9, 10]. In fine-needle aspiration biopsy, the presence of PB strongly suggests malignancy, and PTC in particular [9]. Psammoma bodies are seen in up to 50 % of PTC cases [10] and are considered to be a reliable diagnostic feature [6, 9, 10].

Radiologically, ultrasonographic findings suggesting malignancy in a thyroid nodule include microcalcifications, absence of halos, marked hypoechogenicity, extraglandular extension, an irregular or microlobulated margin, and a heterogeneous echo structure [11–13]. Ultrasonographic calcifications are classified as macrocalcifications or microcalcifications [13]. Although ultrasonographic calcification can be detected in both benign and malignant thyroid nodules, these calcifications represent a potential risk factor for malignancy [12, 14, 15].

The purpose of the present study was to investigate the correlation between the presence of PB and clinicopathological characteristics in conventional PTC ($n = 258$). In addition, we investigated the correlation between ultrasonographic calcification with PB frequency and aggressive PTC behaviors.

Patients and methods

Patients

Two hundred fifty-eight surgically resected conventional PTC obtained from the Department of Pathology, Kangbuk Samsung Hospital, Sungkyunkwan University School of Medicine (Seoul, Korea) from January 1 to December 31, 2010 were analyzed. Among consecutive surgery patients, we excluded patients without ultrasonographic findings. Clinicopathological parameters such as age, gender, tumor size, multifocality, pTNM stage, extrathyroidal extension, lymph node metastasis, and ultrasonographic calcifications were evaluated according to the 7th Edition of the American Joint Cancer Committee (AJCC) TNM classifications [16] by reviewing medical charts, pathological records, and glass slides. All patients underwent preoperative ultrasonography (US) and tumors were confirmed as PTC via fine-needle aspiration biopsy. The mean age of patients at diagnosis was 47.2 years, and patients had undergone either total thyroidectomy ($n = 177$) or hemithyroidectomy ($n = 81$) according to American Thyroid Association guidelines [17]. Central node dissection was performed in all patients, and modified radical neck dissection was selectively performed in patients confirmed with lateral neck nodal metastasis via preoperative fine-needle aspiration biopsy or frozen section ($n = 17$). The mean tumor size was 1.02 ± 0.62 cm. Based on the AJCC stage grouping, 168 cases were classified as stage I, 0 as stage II, 83 as stage III, and 7 as stage IV. This protocol was reviewed and approved by the Institutional Review Board of Kangbuk Samsung Hospital (Approval No. KBC12072).

Evaluation of pathological and radiological features of PTC

Pathological features such as the presence of PB, tumor size, multifocality, extrathyroidal extension, and lymph node metastasis were screened by two pathologists (J.S.P. and G.K.). In our institution, thyroid specimens are longitudinally sectioned, and one section per 0.3 cm of tumor thickness according to the tumor size is submitted for histological examination. At least one section containing peritumoral parenchyma with thyroidal capsule was included for evaluation of extrathyroidal extension. Multifocal tumors may represent either multiple independent primary tumors or intraglandular dissemination from a primary tumor. Regardless of origin, multifocal tumors were defined in this study as tumors that were distant from each other by greater than 0.5 cm. We considered the main tumor as that with the largest size as diagnosed via preoperative and intraoperative examination. In addition, we evaluated the presence of PB and the correlation with ultrasonographic calcification for the main tumor only among multifocal tumors. Based on location, PB are categorized as being within the tumor mass, in the extratumoral tissue, or in the dissected lymph node. The presence of PB was ascertained based on hematoxylin and eosin stained glass slides. Stromal calcifications were not included as evidence of PB. Ultrasonographic evaluations, such as intratumoral calcification, were investigated by radiologists at our institution and reported according to standardized criteria. Ultrasonographic intratumoral calcifications included both microcalcifications (smaller than 0.2 cm) and macrocalcifications (larger than 0.2 cm).

Detection of the $BRAF^{V600E}$ mutation

For the detection of the $BRAF^{V600E}$ mutation, nucleic acid from fresh thyroid tissue from patients who had given informed consent was isolated with a DNA extraction kit (Qiagen, Hilden, Germany) according to the manufacturer's protocol. Isolated nucleic acid was mixed with a polymerase chain reaction (PCR) master mix (4 μL of BRAF PM, 3 μL of 8-Mop solution, and 10 μL of 2× Multiplex Master Mix) from a Seeplex BRAF V600E ACE detection kit (Seegene Inc., Seoul, Korea). Mixed samples were immediately placed in a preheated (94 °C) thermal cycler for 15 min, and the PCR was carried out using the

recommended program in a GeneAmp PCR 9700 system (Applied Biosystems, Foster City, CA). The cycling amplification program was composed of 35 cycles: denaturation for 30 s at 94 °C, annealing for 30 s at 63 °C, and extension for 1 min at 72 °C. The amplified PCR products were loaded on a 2 % agarose gel and were visualized with the SafeView Stain (Applied Biological Materials Inc., Richmond, BC, Canada). The BRAF mutation was detected with a Gel Documentation system (Bio-Rad, Hercules, CA).

Statistical analysis

All statistical analyses were conducted with SPSS version 18.0 software (SPSS, Chicago, IL). The significance of any correlation between the presence of PB and the clinicopathological parameters was determined by either the χ^2 test or Fisher's exact test (two-sided). The relationship between the presence of PB and tumor size was analyzed with a two-tailed Student's t test. Multivariate logistic regression analysis was performed to identify the most influential variables associated with the presence of PB. Results are represented as P values, and odds ratios (OR) with 95 % confidence intervals (CI). The results were considered statistically significant when $P < 0.05$.

Results

The presence of PB is associated with PTC aggressiveness

To evaluate the correlation between the presence of PB and clinicopathological parameters, we reviewed the clinicopathological features of 258 PTC (Table 1). Psammoma bodies were observed in 141 of 258 PTC (54.7 %). The presence of PB was significantly correlated with tumor multifocality, extrathyroidal extension, and lymph node metastasis ($P = 0.009$, $P = 0.004$, and $P < 0.001$, respectively), but not age or gender, by univariate analysis. There was no significant difference in the incidence of PB between $BRAF^{V600E}$ mutated and $BRAF^{V600E}$ non-mutated PTC. However, in multivariate analysis, the presence of PB was an independent predictor of lymph node metastasis ($P = 0.005$, OR = 2.249, 95 % CI 1.272–3.976), but not age, gender, $BRAF^{V600E}$ mutation, tumor multifocality, or extrathyroidal extension (data not shown). According to the AJCC TNM classification [16], the presence of PB was significantly associated with primary tumor (T) stage ($P = 0.008$) and regional lymph node (N) stage ($P < 0.001$). However, the tumor stage grouping showed no difference between the two groups ($P = 0.241$).

Table 1 The correlation between the presence of psammoma bodies (PB) and the clinicopathologic characteristics in papillary thyroid carcinoma

	Psammoma body		P value
	Present	Absent	
Total ($n = 258$)	141 (54.7)	117 (45.3)	
Age, years			
< 45	70 (49.6)	52 (44.4)	0.405
≥ 45	71 (50.4)	65 (55.6)	
Gender			
Male	31 (22.0)	32 (18.8)	0.529
Female	110 (78.0)	95 (81.2)	
Tumor size	1.16 ± 0.65 cm	0.85 ± 0.54 cm	0.009
BRAF mutation			
Present	114 (80.9)	93 (79.5)	0.784
Absent	27 (19.1)	24 (20.5)	
Multifocality of tumor			
Yes	51 (36.2)	25 (21.4)	0.009
No	90 (63.8)	92 (78.6)	
Extrathyroidal extension			
Yes	90 (63.8)	54 (46.2)	0.004
No	51 (36.2)	63 (53.8)	
Lymph node metastasis			
Yes	87 (61.7)	40 (34.2)	<0.001
No	54 (38.3)	77 (65.8)	
Primary tumor (T)			
T1	50 (35.5)	64 (54.7)	0.008
T2	1 (0.7)	1 (0.9)	
T3	90 (63.8)	52 (44.4)	
T4	0 (0.0)	0 (0.0)	
Regional lymph nodes (N)			
N0	54 (38.3)	77 (65.8)	<0.001
N1a	73 (51.8)	37 (31.6)	
N1b	14 (9.9)	3 (2.6)	
Tumor stage			
I	89 (63.1)	79 (67.5)	0.241
II	0 (0.0)	0 (0.0)	
III	46 (32.6)	37 (31.6)	
IV	6 (4.3)	1 (0.9)	

Numbers in parentheses represent percentages

Tumor size is associated with the incidence of PB

Although lower rates of extrathyroidal extension and nodal metastasis have been shown with papillary microcarcinomas (PMC) (≤1 cm) than with overt PTC (>1 cm), little is known about the correlation between tumor size and the presence of PB [6, 18]. Thus, we investigated the correlation between the incidence of PB and tumor size (>1 vs. ≤1 cm). The mean tumor sizes of PTC with PB and those

without PB were 1.16 ± 0.65 and 0.85 ± 0.54 cm, respectively ($P = 0.009$) (Table 1). Psammoma bodies were found more frequently in overt PTC than in PMC ($P < 0.001$). In addition, in overt PTC, higher incidences of PB were found in intratumoral and extratumoral regions and lymph nodes compared with PMC ($P < 0.001$, $P = 0.015$ and $P = 0.002$, respectively) (Table 2).

Extratumoral PB are associated with more aggressive PTC behaviors than intratumoral PB

Among the 141 PTCs that contained PB, extratumoral PB were found in 69 PTCs and only intratumoral PB were in the remaining 72 PTCs. Seven of the 69 PTC with extratumoral PB were detected only in extratumoral PB without intratumoral PB. The presence of extratumoral PB was significantly correlated with tumor multifocality, extrathyroidal extension, and lymph node metastasis compared with the presence of intratumoral PB ($P = 0.014$, $P = 0.005$, and $P = 0.001$, respectively) (Table 3). However, PTC with intratumoral PB, regardless of the presence of extratumoral PB, were also significantly correlated with tumor multifocality, extrathyroidal extension, and lymph node metastasis compared to PTC without PB ($P = 0.040$, $P = 0.002$, and $P < 0.001$, respectively) (data not shown).

Table 2 The correlation between the presence of PB and tumor size of papillary thyroid carcinoma

	PMC (≤1.0 cm)	Overt PTC (>1.0 cm)	*P* value
Total ($n = 258$)	162 (62.8)	96 (37.2)	
Psammoma body			
Present	74 (45.7)	67 (69.8)	<0.001
Absent	88 (54.3)	29 (30.2)	
Intratumoral psammoma body			
Present	69 (42.6)	65 (67.7)	<0.001
Absent	93 (57.4)	31 (32.3)	
Extratumoral psammoma body			
Present	35 (21.6)	34 (35.4)	0.015
Absent	127 (78.4)	62 (64.6)	
Psammoma body in lymph node			
Present	42 (25.9)	43 (44.8)	0.002
Absent	120 (74.1)	53 (55.2)	
Ultrasonographic intratumoral calcification			
Present	65 (40.1)	54 (56.3)	0.012
Absent	97 (59.9)	42 (43.7)	

Numbers in parentheses represent percentages. Overt PTC describes PTC above 1 cm

PMC papillary microcarcinoma

Table 3 The correlation between the presence of intratumoral and extratumoral PB and clinicopathologic characteristics in papillary thyroid carcinoma

	Intratumoral PB	Extratumoral PB	*P* value
Total ($n = 141$)	72 (51.1 %)	69 (48.9 %)	
Multifocality of tumor			
Yes	19 (26.4 %)	32 (46.4 %)	0.014
No	53 (73.6 %)	37 (53.6 %)	
Extrathyroidal extension			
Yes	38 (52.8 %)	52 (75.4 %)	0.005
No	34 (47.2 %)	17 (24.6 %)	
Lymph node metastasis			
Yes	35 (48.6 %)	52 (75.4 %)	0.001
No	37 (51.4 %)	17 (24.6 %)	

Ultrasonographic intratumoral calcifications are associated with higher incidences of PB and lymph node metastasis

Little is known about the correlation between ultrasonographic calcification and the presence of PB in PTC. Thus, we investigated the correlation between ultrasonographic intratumoral calcifications and the presence of PB. In the present study, ultrasonographic intratumoral calcification included intratumoral microcalcification and macrocalcification, but not rim calcification, based on the radiologic records. Among the 258 PTC studied, ultrasonographic intratumoral calcifications were detected in 119 (46.1 %). Although intratumoral PB were found in both the ultrasonographic intratumoral calcifications detected and non-detected groups, the incidence of intratumoral PB was significantly increased in the detected group (64.7 %) compared with the non-detected group (41.0 %) ($P < 0.001$) (Table 4). Our data also showed a significantly increased frequency of lymph node metastasis in PTC with ultrasonographic intratumoral calcifications ($P = 0.026$) (Table 4). The frequency of extrathyroidal extension was slightly increased in PTC with ultrasonographic intratumoral calcification ($P = 0.098$). When evaluated by tumor size, the presence of PB showed significant correlations with ultrasonographic intratumoral calcifications and lymph node metastasis in PMC ($P = 0.001$ and $P = 0.003$, respectively), even though, as shown in Table 2, ultrasonographic intratumoral calcifications showed a higher incidence in overt PTC (56.3 %) than in PMC (40.1 %) ($P = 0.012$).

Discussion

Although PB are found in up to 50 % of PTC, their clinicopathological significance has not been fully elucidated.

Table 4 The correlation between the ultrasonographic intratumoral calcifications and the presence of psammoma bodies, extrathyroidal extension, and lymph node metastasis in papillary thyroid carcinoma

	Ultrasonographic intratumoral calcification		*P* value
	Present	Absent	
Total ($n = 258$)	119 (46.1)	139 (53.9)	
Intratumoral psammoma body			
Present	77 (64.7)	57 (41.0)	<0.001
Absent	42 (35.3)	82 (59.0)	
Extrathyroidal extension			
Present	73 (61.3)	71 (51.1)	0.098
Absent	46 (38.7)	68 (48.9)	
Lymph node metastasis			
Present	67 (56.3)	59 (42.4)	0.026
Absent	52 (43.7)	80 (57.6)	

Numbers in parentheses represent percentages

In a previous study, the presence of intratumoral PB was found to be associated with gross lymph node metastasis, but not extrathyroidal extension or histological lymph node metastasis [6]. However, due to exclusion of PMC in that study, it could not be concluded whether the presence of PB has an effect on the tumor behaviors of PTC. The present study included both PMC and overt PTC, and it showed that the presence of PB was significantly correlated with tumor multifocality, extrathyroidal extension, and lymph node metastasis by univariate analysis (Table 1). These results suggest that the presence of PB may be useful in predicting aggressive tumor behaviors in PTC patients.

The most common form of intrathyroidal calcification observed by thyroid US is the coarse, dense, and nodular variety [19]. Microcalcifications, representing clusters of PB, were found in up to 50 % of thyroid cancer cases [13, 19–21]. Moon et al. [13] reported that microcalcification and macrocalcification, but not rim calcification, were found more frequently in malignant nodules than in benign nodules. However, little is known about the correlation between ultrasonographic intratumoral calcification and the presence of intratumoral PB. In the present study, ultrasonographic intratumoral calcifications were significantly associated with a high incidence of intratumoral PB (Table 4). Taken together, our results suggest that the detection of ultrasonographic intratumoral calcification is a potential indicator of the presence of PB.

Cervical lymph nodes are classified into central and lateral compartments. Cervical lymph node metastasis occurs in 30–80 % of PTC cases [22, 23]. Although US has been reported to be the most sensitive and useful method for the detection of metastatic lymph nodes in PTC, preoperative US cannot detect all metastatic nodes because of the small size of the nodes and the problematic anatomy of the neck [24]. Ito and Miyauchi [25] reported that, while metastatic lateral lymph nodes were undetectable by preoperative US, lateral lymph node metastasis was detected microscopically in more than half of these cases. In the present study, 65 of 126 PTC with nodal metastasis were suspicious by preoperative US and only 51 of 65 cases were confirmed by pathologic examination. Among 193 PTC with no ultrasonographic nodal metastasis suspicion, those with ultrasonographic intratumoral calcification showed a higher incidence of nodal metastasis than those with no ultrasonographic intratumoral calcification ($P < 0.001$) (data not shown). Therefore, our results suggest that ultrasonographic intratumoral calcification together with US suspicious feature of nodal metastasis would be a useful decision-making criterion regarding the need for surveillance of suspicious metastatic lymph nodes.

Psammoma bodies may be formed by necrosis and calcification of intravascular or intralymphatic tumor thrombi and by intracellular calcifications in the viable cells of the nidus [6]. It has been speculated that extratumoral PB are associated with the spread of tumor cells via extratumoral vascular or lymphatic channels. However, there have been no previous studies regarding the clinicopathological significance of extratumoral PB in PTC. The presence of extratumoral PB was significantly correlated with higher incidences of tumor multifocality, extrathyroidal extension, and lymph node metastasis, compared with the presence of intratumoral PB (Table 3). Thus, our results suggest that extratumoral PB are associated with aggressive tumor behaviors.

Wang et al. [14] reported that the incidence of ultrasonographic calcification in PMC is similar to that in overt PTC. However, in the present study, ultrasonographic intratumoral calcifications showed a higher incidence in overt PTC than in PMC (Table 2). This discrepancy with previous studies may stem from the difference in the total number of patients and the composition of PMC and overt PTC. Although overt PTC showed greater frequencies of PB and ultrasonographic intratumoral calcification, the presence of PB in PMC was also significantly associated with lymph node metastasis and ultrasonographic intratumoral calcification. However, tumor multifocality and extrathyroidal extension were not significantly correlated with the presence of PB in overt PTC or PMC (data not shown). Our data suggest that the presence of PB is strongly correlated with lymph node metastasis and ultrasonographic intratumoral calcification.

Until recently, little information existed regarding the relationship between the presence of PB and the $BRAF^{V600E}$ mutation. Our data revealed no significant correlation between PB and the $BRAF^{V600E}$ mutation. In the present study, the $BRAF^{V600E}$ mutation showed high frequency up to 90 %, consistent with previous reports [26, 27]. Due to the

high frequency of the $BRAF^{V600E}$ mutation, it could be limited to evaluate the correlation between the incidence of PB and $BRAF^{V600E}$ mutation. Although the mechanism of PB formation is not fully understood, our data suggest that factors affecting tumor growth rather than the $BRAF^{V600E}$ mutation may be associated with the formation of PB. Further study of a possible correlation between PB formation and the $BRAF^{V600E}$ mutation is needed.

In the present study, although the presence of PB was significantly correlated with primary tumor (T) and regional lymph node (N) stages, there was no significant correlation with stage groupings. In the AJCC TNM classification [16], PTC are divided based on patient age (under 45 or 45 years and older), and separate stage grouping rules are applied. Patients under 45 years old are categorized as stage I or stage II, depending on the presence of distant metastasis, and all patients in the present study were classified as stage I. In patients 45 years and older, PTC without advanced disease are classified as stage I to IV by the definitions of T and N staging. These characteristics of AJCC TNM classification may affect the discrepancy observed between tumor stage grouping and tumor behaviors.

In conclusion, the results of the present study suggest that the presence of PB may indicate aggressive behaviors such as tumor multifocality, extrathyroidal extension, and lymph node metastasis. In addition, the detection of preoperative ultrasonographic calcification could be a useful predictor of the presence of PB and lymph node metastasis.

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