

Evaluation of cytopathological findings in thyroid nodules with macrocalcification: macrocalcification is not innocent as it seems

Avaliação dos achados citopatológicos em nódulos tireoidianos com macrocalcificações: elas não são tão inocentes como parecem

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ABSTRACT

Objective: Microcalcification is strongly correlated with papillary thyroid cancer. It is not clear whether macrocalcification is associated with malignancy. In this study, we aimed to assess the result of fine needle aspiration biopsies (FNAB) of thyroid nodules with macrocalcifications. **Subjects and methods:** We retrospectively evaluated 269 patients (907 nodules). Macrocalcifications were classified as eggshell and parenchymal macrocalcification. FNAB results were divided into four groups: benign, malignant, suspicious for malignancy, and non-diagnostic. **Results:** There were 79.9% female and 20.1% male and mean age was 56.9 years. Macrocalcification was detected in 46.3% nodules and 53.7% nodules had no macrocalcification. Parenchymal and eggshell macrocalcification were observed in 40.5% and 5.8% nodules, respectively. Cytologically, malignant and suspicious for malignancy rates were higher in nodules with macrocalcification compared to nodules without macrocalcification ($p = 0.004$ and $p = 0.003$, respectively). Benign and non-diagnostic cytology results were similar in two groups ($p > 0.05$). Nodules with eggshell calcification had higher rate of suspicious for malignancy and nodules with parenchymal macrocalcification had higher rates of malignant and suspicious for malignancy compared to those without macrocalcification ($p = 0.01$, $p = 0.003$ and $p = 0.007$, respectively). **Conclusions:** Our findings suggest that macrocalcifications are not always benign and are not associated with increased nondiagnostic FNAB results. Macrocalcification, particularly the parenchymal type should be taken into consideration. *Arq Bras Endocrinol Metab.* 2014;58(9):939-45

Keywords

Thyroid nodule; macrocalcification; malignancy; suspicious of malignancy

RESUMO

Objetivo: A microcalcificação está fortemente correlacionada com o câncer papilar de tireoide. Não está claro se a macrocalcificação também está associada com malignidade. Neste estudo, nosso objetivo foi avaliar o resultado da biópsia de aspiração por agulha fina (FNAB) de nódulos tireoidianos com macrocalcificações. **Sujeitos e métodos:** Avaliamos retrospectivamente 269 pacientes (907 nódulos). As macrocalcificações foram classificadas como periféricas (casca de ovo) ou parenquimatosas (interna). Os resultados da FNAB foram divididos em quatro grupos citológicos: benignos, com malignidade, suspeita de malignidade e não diagnósticos. **Resultados:** Das amostras, 79,9% foram coletadas de mulheres e 20,1% de homens, e a idade média foi de 56,9 anos. A macrocalcificação foi detectada em 46,3% dos nódulos, e em 53,7% dos nódulos não havia macrocalcificação. A macrocalcificação parenquimatosa e periférica foi observada em 40,5% e 5,8% dos nódulos, respectivamente. Em termos citológicos, a malignidade e suspeita de malignidade foram mais comuns em nódulos com macrocalcificação em comparação com nódulos sem macrocalcificação ($p = 0,004$ e $p = 0,003$, respectivamente). Resultados benignos e não diagnósticos da citologia foram similares em ambos os grupos ($p > 0,05$). Os nódulos com calcificações periféricas apresentaram uma taxa maior de suspeita de malignidade e os nódulos com macrocalcificação parenquimatosa apresentaram taxas maiores de malignidade e suspeita de malignidade em comparação com nódulos sem macrocalcificação ($p = 0,01$, $p = 0,003$ e $p = 0,007$, respectivamente). **Conclusões:** Nossos achados sugerem que as macrocalcificações não são sempre benignas e esses nódulos não estão associados com maiores resultados não diagnósticos da FNAB. A macrocalcificação, particularmente do tipo parenquimatosa, deve ser levada em consideração. *Arq Bras Endocrinol Metab.* 2014;58(9):939-45

Descritores

Nódulo tireoidiano; macrocalcificação; malignidade; suspeita de malignidade

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INTRODUCTION

Thyroid nodules are commonly observed in the adult population and the incidence is increasing largely related with widespread use of Doppler ultrasonography (US) and other imaging techniques. Around 4-8% of thyroid nodules are found incidentally in asymptomatic adults, whereas 10-41% are detected by US (1). The majority of thyroid nodules are benign, with malignancy rates ranging from 9 to 13% in different studies (2,3). Thyroid US has an important role in the diagnosis of thyroid nodules because it is a simple, non-invasive, effective, and useful method. Nodules with a diameter of 2-3 millimeter (mm) can be detected by high resolution images. Also, vascularity can be determined by colour Doppler or power Doppler US. The use of US and US guided fine needle aspiration biopsy (FNAB) to assess thyroid nodules has reduced the number of unnecessary surgeries and increased the rate of diagnosis of thyroid cancer (4,5). The rate of accuracy of FNAB was reported up to 96%. Morphological features of nodules such as echogenicity, texture, margin regularity, presence of halo, presence and type of calcification can be assessed by high resolution US. Margin irregularity, hypoechoogenicity and microcalcification were considered to be important risk factors for malignancy, however, size of thyroid nodule alone was not considered as a risk factor (6).

Thyroid nodular calcifications can be classified according to their diameter and location; calcifications < 2 mm and without acoustic shadow at posterior are microcalcifications, calcifications \geq 2 mm and with posterior acoustic shadow are macrocalcifications, and calcifications surrounding the nodule are peripheral (eggshell) calcifications. Pathologically, microcalcification is a psammoma body that contains 10-200 μ m, rough, smooth, bright, calcific aggregations (7). Large and irregular bordered macrocalcification can exist secondary to tumor necrosis and it can be seen in both benign and malignant nodules (2,8). Peripheral calcifications are believed to occur secondary to chronic degenerative changes.

Although, microcalcifications are known to be strongly associated with malignant nodules, the association of macrocalcifications with malignancy is controversial (8-15). Recent studies have revealed a relationship between macrocalcification and malignancy, particularly in papillary thyroid carcinomas (9,10,16-18). In addition, despite the general belief that peripheral macrocalcification indicates benign situations, it

was shown that if it is irregular it can also be related with malignancy (8,19). Macrocalcification together with microcalcification in the same nodule or located in the middle of a hypoechoic nodule have a higher probability of malignancy (20).

The role of FNAB in thyroid nodules with macrocalcifications is unclear with 11 to 25% of the biopsies yielding false negative and 5 to 30% yielding non-diagnostic cytologies (19-22). Calcified lesions detected by USG have been reported to be the most common cause of insufficient FNAB sampling (23). In this study, we aimed to evaluate FNAB results of thyroid nodules with parenchymal and peripheral macrocalcifications. We also tried to find out the impact of macrocalcifications on nondiagnostic cytology results.

SUBJECTS AND METHODS

We retrospectively evaluated 907 nodules from 269 patients seen in our out-patient clinic. Patients > 15 years of age with nodular or multinodular goiter and macrocalcification in at least one nodule were included. Patients with a previous history of thyroid surgery, percutaneous invasive procedures for nodules, radiotherapy to head and neck region or radioactive iodine therapy were excluded from the study. Preoperative thyroid functions, thyroid autoantibodies, thyroid US findings and FNAB results were obtained from medical records. The study was approved by the local ethical committee in accordance with the ethical standards of Helsinki declaration.

Blood samples were obtained between 08:00 to 10:00 in the morning from all patients. Serum sensitive thyrotrophin (TSH), free triiodothyronine (fT3), free thyroxine (fT4) and thyroid autoantibodies [antithyroid peroxidase antibody (anti-TPO) and anti-thyroglobulin antibody (anti-TGAb)] levels were measured with chemiluminescent immunoassay (Immulite 2000, Diagnostic Products Corporation, Los Angeles, CA, USA, and the UniCel DxI 800, Beckman Coulter, CA, USA). Normal levels were as follows; TSH: 0.4 - 4.0 uIU/mL, fT3: 1.57 - 4.71 pg/mL, fT4: 0.61 - 1.12 ng/dL, anti-TPO < 10 U/mL and anti-Tg < 30 U/mL.

US was performed with a color Doppler ultrasonography (FCW Tecnology Co., Ltd. Model: 796FDII Yung-ho City, Taipei, Taiwan) and a superficial probe (Esaote, Model No: LA523, 13 - 4, from 5.5 - 12.5 MHz) in all patients. Nodule location, diameters, volume, echogenicity (isoechoic, hypoechoic or hyperechoic), texture (solid, mixed or cystic), marginal

regularity (regular or irregular), presence of hypoechoic halo, presence and type of calcification (microcalcification, parenchymal macrocalcification, peripheral macrocalcification) and vascularization pattern were recorded for all nodules evaluated with FNAB. We defined calcifications < 2 mm as microcalcification and ≥ 2 mm in diameter and with an acoustic shadow as macrocalcification (Figure 1).

Thyroid FNAB was performed by an experienced clinician with 27-gauge needle and 20 mL syringe under US guidance. Each nodule was aspirated for 2 - 4 times and at least 4 - 6 preparations were obtained from each aspiration. Cytological assessment was conducted by an experienced cytopathologist. FNAB materials were air-dried and stained by May-Grunwald-Giemsa. The cytological diagnoses were classified as benign, non-diagnostic, suspicious for malignancy and malignant. FNAB results of nodules with parenchymal and peripheral macrocalcifications were compared with nodules not including macrocalcification in the same patient group.

All the data were analyzed with SPSS (Statistical Package of Social Science for Windows) 15.0. Descrip-

tive statistics were expressed as mean \pm standard deviation for continuous variables and as number of cases and percentage for nominal variables. Student's *t* test was used to compare differences between independent groups for continuous variables and Chi-square test was used to compare nominal variables. A *p* value < 0.05 was considered statistically significant.

RESULTS

There were 215 female (79.9%) and 54 (20.1%) male patients and the mean age was 56.9 ± 13.1 years (21 - 87 years). One hundred and sixty-one (60%) patients had multinodular goiter and 108 (40%) patients had solitary thyroid nodule. Macrocalcifications were observed in 420 (46.3%) nodules, and 487 (53.7%) nodules had no macrocalcification. Parenchymal and peripheral macrocalcifications were present in 367 (40.5%) and 53 (5.8%) of 907 nodules, respectively. Mean diameters of nodules with macrocalcification and without macrocalcification were 23.92 ± 14.15 mm and 15.72 ± 7.53 mm, respectively ($p < 0.001$) (Table 1).

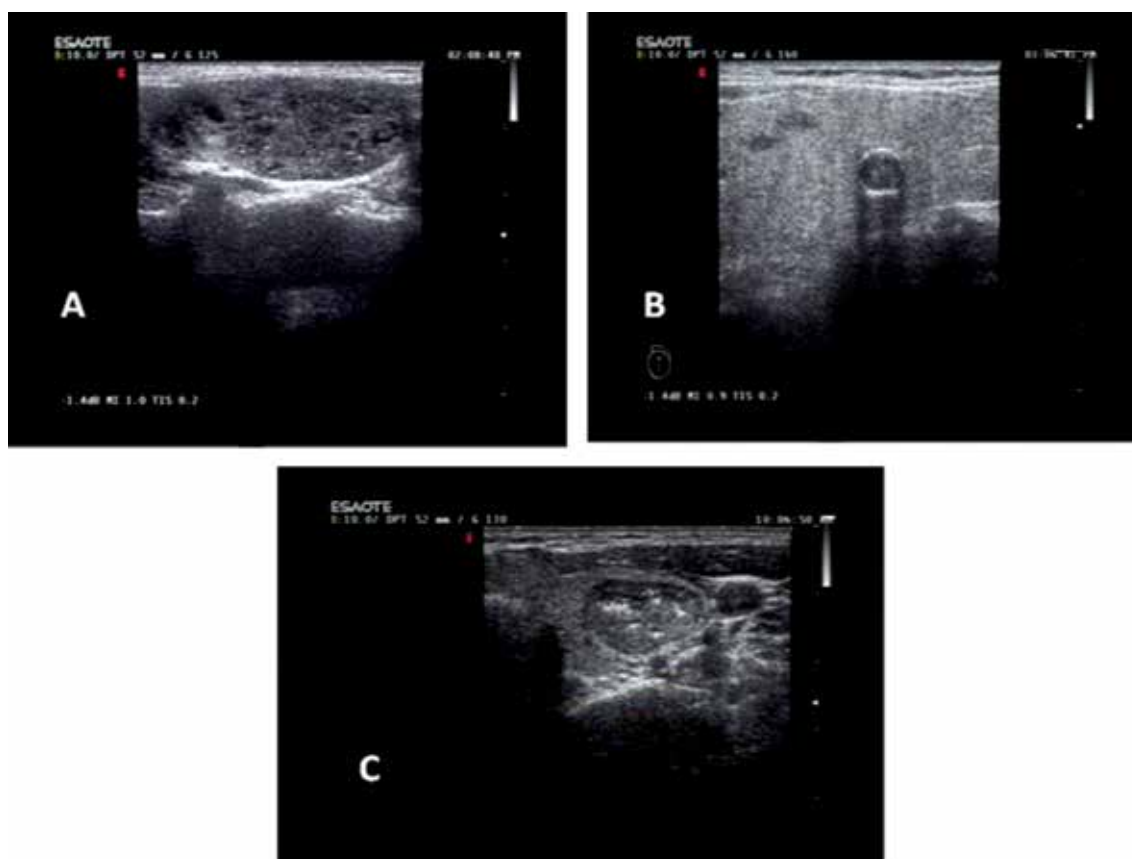


Figure 1. Thyroid nodule calcifications detected in ultrasonography. (A) Microcalcification, (B) peripheral (eggshell) macrocalcification, (C) parenchymal (internal) macrocalcification.

Table 1. Ultrasonography features and cytological results of thyroid nodules with and without macrocalcification

	Nodules with macrocalcification (n = 420) (%)	Nodules without macrocalcification (n = 487) (%)	p
Ultrasonography features			
Nodule diameter (mm)	23.92 ± 14.15	15.72 ± 7.53	< 0.001
Nodule volume (mL)	8.53 ± 13.21	2.25 ± 3.93	< 0.001
Presence of halo	97 (23.1)	111 (22.8)	0.914
Microcalcification	258 (61.4)	85 (17.5)	< 0.001
Margin regularity	141 (33.6)	171 (35.1)	0.626
Regular	279 (66.4)	316 (64.9)	
Irregular			
Echogenity			
Hypoechoic	136 (32.4)	217 (44.6)	
Isoechoic	281 (66.9)	266 (54.6)	< 0.001
Hyperechoic	3 (0.7)	4 (0.8)	
Texture			
Solid	49 (11.7)	243 (49.9)	
Cystic	1 (0.2)	10 (2.1)	< 0.001
Mixed	370 (88.1)	234 (48)	
Cytological result			
Benign	316 (75.2)	391 (80.3)	0.067
Malignant	15 (3.6)	4 (0.8)	0.004
Suspicious for malignancy	23 (5.5)	9 (1.9)	0.003
Non-diagnostic	66 (15.7)	83 (17)	0.590

Nodules with macrocalcification had significantly higher volume compared to nodules without macrocalcification. Ultrasonographically, rates of presence of hypoechoic halo and margin regularity were similar in two groups. Microcalcifications were observed more commonly in nodules with macrocalcification ($p < 0.001$). Thirty-two point four percent of nodules with macrocalcification and 44.6% of nodules without macrocalcification were hypoechoic ($p < 0.001$). In terms of texture, nodules with macrocalcification had a higher prevalence of solid-cystic mixed texture, while nodules without macrocalcification had a higher prevalence of solid texture (Table 1).

Cytological results of 420 nodules with macrocalcification were benign in 75.2%, non-diagnostic in 15.7%, suspicious for malignancy in 5.5% and malignant in 3.6%. Of the nodules without macrocalcification, 80.3% were benign, 0.8% malignant, 1.9% suspicious for malignancy, and 17% non-diagnostic (Table 1). Accordingly, the rates of suspicious for malignancy and

malignant results were significantly higher in nodules with macrocalcification compared to nodules without macrocalcification ($p = 0.004$ and $p = 0.003$, respectively).

When we compared cytological results of nodules with peripheral macrocalcification and without macrocalcification, we found that the rate of suspicious for malignancy was higher in nodules with peripheral macrocalcification while rate of benign was higher in nodules without macrocalcification ($p = 0.01$ and $p = 0.036$, respectively) (Table 2). Cytologically, 3.8% of nodules with parenchymal macrocalcification and 0.8% of nodules without macrocalcification were malignant ($p = 0.003$). Suspicious for malignancy rate was also higher in nodules with parenchymal macrocalcification compared to nodules without macrocalcification ($p = 0.007$) (Table 3). Although rate of nondiagnostic cytology was higher in nodules with peripheral macrocalcification, the difference was not statistically significant. In multiple logistic regression analysis, macrocalcification was found to be related with malignant cytology results independent from presence of microcalcification, irregular margins and absence of halo ($p = 0.008$).

The numbers and rate of the thyroid nodules with or without micro/macro-calcifications determined as suspicious for malignancy or malignant were shown in the table 4.

Table 2. Cytological results of thyroid nodules with peripheral macrocalcification and without macrocalcification

Cytological result	Nodules with peripheral macrocalcification (n = 53) (%)	Nodules without macrocalcification (n = 487) (%)	p
Benign	67.9	80.3	0.036
Malignant	1.9	0.8	0.442
Suspicious for malignancy	7.5	1.9	0.010
Non-diagnostic	22.7	17	0.309

Table 3. Cytological results of thyroid nodules with parenchymal macrocalcification and without macrocalcification

Cytological result	Nodules with parenchymal macrocalcification (n = 367) (%)	Nodules without macrocalcification (n = 487) (%)	p
Benign	76.3	80.3	0.159
Malignant	3.8	0.8	0.003
Suspicious for malignancy	5.2	1.9	0.007
Non-diagnostic	14.7	17	0.359

Table 4. The numbers and rate of the thyroid nodules with or without micro/macro-calcifications determined as suspicious for malignancy or malignant

	Suspicious for malignancy	Malignant
Nodules with macrocalcification/with microcalcification (n = 258)	16 (6.2%)	11 (4.3%)
Nodules with macrocalcification/without microcalcification (n = 162)	7 (4.4%)	4 (2.5%)
Nodules without macrocalcification/without microcalcification (n = 402)	7 (1.7%)	4 (1%)
Nodules without macrocalcification/with microcalcification (n = 85)	2 (2.3%)	0 (0%)

Histopathological results were available in 43 patients who underwent surgery for various reasons such as malignant or suspicious for malignancy cytology results, giant nodule, compression symptoms and suspicious US findings. There were 18 patients with malignant and 25 patients with benign histopathology. Ultrasonographically, micro and macrocalcification, particularly parenchymal macrocalcification were more prevalent in malignant nodules compared to benign nodules (Table 5).

Table 5. Preoperative calcification types in ultrasonography in patients with final histopathological results

Calcification type	Malignant (n = 18) (%)	Benign (n = 25) (%)	p
Microcalcification	14 (77.7)	12 (48)	0.049
Macrocalcification	13 (72.2)	10 (40)	0.037
Peripheral macrocalcification	2 (11.1)	2 (8)	0.473
Parenchymal macrocalcification	13 (72.2)	9 (36)	0.019
Without macrocalcification	2 (11.1)	11 (44)	0.021

DISCUSSION

Microcalcification in thyroid nodules is known to be associated with malignancy; however, the relationship between macrocalcification and malignancy is controversial. Large calcifications with irregular borders may occur secondary to tumor necrosis and may be present

in benign and malignant nodules (7,18). Previously, peripheral calcification was thought to occur secondary to chronic degenerative changes and therefore indicate a benign status. However, recent studies have found that macrocalcifications including peripheral calcification might also be an indicator of thyroid nodule malignancy (9,10,16-18,24,25). In this study, we showed that cytologically malignant and suspicious for malignancy results are observed more frequently in nodules with parenchymal macrocalcification. Also, nodules with peripheral macrocalcification had a higher rate of suspicious for malignancy results.

Taki and cols. assessed preoperative US findings in 151 surgically resected thyroid nodules and found that 57 (38%) of nodules had calcification (14). Among 11 nodules with microcalcification, 9 (82%) were malignant and among 46 nodules with macrocalcification (intranodular and peripheral) 22 (47.8%) were malignant. Additionally, malignancy was histologically identified in 6 (43%) of 14 nodules with peripheral calcification. The authors concluded that all calcification types may be associated with malignancy and nodules with macrocalcification should be examined thoroughly.

In previous studies, histopathologically proven malignancy rate of thyroid nodules with peripheral macrocalcification was reported to range between 18.5% to 70% with most of studies showing higher than 50% malignancy rate in these nodules (8-10,23). Majority of carcinomas were papillary type, with a few follicular carcinoma histopathologically. Even, anaplastic carcinoma was reported in nodules with peripheral macrocalcification which was blamed for insufficient FNAB result (26). Although there are some US criteria known to be associated with malignancy, it is difficult to apply these criteria for nodules with peripheral macrocalcification due to posterior shadowing and inability to interpret marginal regularity. This has led to search for additional criteria to indicate malignancy in these nodules. In the study by Park and cols., thickening and interruption of peripheral calcifications were suggested to be significant indicators of malignancy (11).

Ugurlu and cols. (27) retrospectively assessed the FNAB results of 1,004 patients with thyroid nodules and found that the risk of malignancy was greater in nodules containing microcalcification than those without calcification. However, presence of macrocalcification was not associated with increased risk of malignancy in FNAB compared to nodules without macrocalcification. These results are contrary to our

findings and those of some previous studies. We have observed cytologically higher malignant and suspicious for malignancy rates in nodules with macrocalcification compared to those without macrocalcification. Similarly, in a recent trial including 713 subcentimeter nodules, solid composition and macrocalcification in addition to hypoechogenicity, infiltrative margin, microcalcification, and taller-than-wide shape were found to be significantly associated with malignant cytology (28). The authors showed that including solid composition with or without macrocalcification improved the diagnostic performance in subcentimeter nodules for the identification of malignant lesions. Park and cols., investigated sonographic findings of 854 macrocalcified nodules and reported that 171 (20.8%) were nondiagnostic cytologically, 470 (55.0%) were benign (18 were confirmed by histopathology) and 179 (20.9%) were malignant histopathologically (29). In that study, the rates of nondiagnostic and suspicious for malignancy cytologies were similar with our findings. However, rate of malignancy was higher and rate of benign result was lower compared to our study. As the authors have mentioned as a limitation of their study, patients with benign findings at US had not undergone biopsy or surgery which might have resulted in relatively fewer benign nodules.

In contrary to some previous reports suggesting that the presence of calcification is significantly associated with non-diagnostic FNAB cytology (30), we found no difference in terms of non-diagnostic cytology between nodules with or without macrocalcification. This finding was also supported in a recent trial by Lee and cols. who retrospectively reviewed sonographic findings and histopathological results of 188 nodules with macrocalcification (23). They showed that 6.9% of nodules with macrocalcification was nondiagnostic cytologically and sensitivity, specificity, positive predictive value and negative predictive value of FNAB were all higher than 90% with a diagnostic accuracy of 96% in these nodules. The authors suggested that FNA of thyroid nodules with macrocalcification had a high diagnostic yield. In another study, ultrasonographic features of 1,195 nodules with inadequate cytology were evaluated prospectively and neither micro- nor macrocalcification was reported to be related with increased risk of inadequacy (31).

Our study has several limitations including the retrospective design and the fact that histopathological results were available only in a small percentage of

patients who underwent surgery. Thus, we could not determine the exact effect of macrocalcification on false positivity or negativity of FNAB in nodules with macrocalcification.

In conclusion, peripheral and parenchymal macrocalcifications are associated with higher suspicious for malignancy and/or malignant results in FNAB. In addition to hypoechogenicity, marginal irregularity, absence of halo and vascularization pattern, the presence of macrocalcification in a nodule might be accepted as one of the suspicious US features. However, further studies including histopathological confirmation of these cytological findings are required to support this suggestion. Also, presence of macrocalcification is not related with increased nondiagnostic cytology in FNAB and should not prevent clinicians from making further assessments in case of nondiagnostic results.

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