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Combination of thyroid ultrasound examination (TIRADS) and survivin gene mRNA expression to determine the type of thyroid nodule

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ABSTRACT

Background: Ultrasound plays a significant role in determining the diagnosis of thyroid nodules. The thyroid ultrasound examination is operator dependent, so the Thyroid Imaging Reporting and Data System (TIRADS) method were developed. However, until now TIRADS has not been able to replace the Fine Needle Aspiration (FNA) examination in determining the type of benign or malignant thyroid nodule, so it is necessary to find alternative biomarkers that can be used to increase the sensitivity and specificity of TIRADS. In this study, the mRNA expression of the survivin gene in thyroid nodules was examined in combination with TIRADS so that it is expected to increase the diagnostic value.

Method: This study is a diagnostic test with a cross sectional design on 51 patients with thyroid nodules in 5 hospitals on the island of Lombok. Subjects who met the inclusion and exclusion criteria were subjected to TIRADS examination using an ultrasound machine and mRNA expression of the survivin gene using cubital vein blood samples with polymerase chain reaction (PCR).

Result: The characteristics of the research subjects were dominated by female sex with age 40 years, TIRADS 3 and histology of benign thyroid nodules. Analysis using the ROC curve obtained TIRADS has a sensitivity of 94.4%, specificity 39.4%, accuracy 58.82%, Positive Predictive Value (PPV) 45.9%, Negative Predictive Value (NPV) 92.9%. While Survivin has 100% sensitivity, 60.6% specificity, 58.1% accuracy, 58.1% PPV, 100% NPV. In the serial combination of TIRADS and survivin, there was an increase in the diagnostic value, namely sensitivity 94.4%, specificity 75.8%, accuracy 82.35%, PPV 68%, NPV 96.2%.

Conclusion: The combination of TIRADS and survivin can increase the diagnostic value in determining the type of thyroid nodule.

Keywords: TIRADS, survivin, diagnostic test, accuracy.
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INTRODUCTION

Thyroid nodules are found in 60% of the human population. Most thyroid nodules are benign, whereas malignant one account for only about 10% of cases.[1] The prevalence of thyroid nodules by palpation is about 5%, whereas using ultrasound 65%, Computed Tomography (CT)-Scan or Magnetic Resonance Imaging (MRI) is about 15% while using Fluorodyoxy Glucose Positron Emission Tomography (PET) is about 2% so in practice ultrasound plays a significant role in determining the diagnosis of thyroid nodules. The disadvantage of using ultrasound is that it is operator-dependent. In an effort to achieve uniform results from "operator-dependent" ultrasound examination of thyroid nodules, the Thyroid Imaging Reporting and Data System (TIRADS) scoring method was developed.[2] Research conducted by Hovath et al.[3] using the TIRADS scoring system obtained 88% sensitivity, 49% specificity, 49% Positive Predictive Value (PPV), 88% Negative Predictive Value (NPV), and 94% accuracy. This study used a sample of 1097 thyroid nodules compared with the results of Fine Needle Aspiration (FNA). Research conducted using TIRADS scoring has not been able to replace the FNA examination in determining benign or malignant thyroid nodules, so it is necessary to find an alternative biomarker that can be used to increase the sensitivity and specificity of the TIRADS and hopefully reduce unnecessary FNA tests. In this study, the m-RNA expression of the survivin gene was examined in both benign and malignant thyroid nodules, so that the expected results were differences in the m-RNA expression of the survivin gene in benign and malignant thyroid nodules. Then the cut-off value of each

m-RNA expression of the survivin gene was determined in predicting benign and malignant thyroid nodules. These results can be used as an alternative choice when combined with TIRADS so that it is hoped that the diagnostic value can increase.

METHOD

Study design and participant

This study is a diagnostic test study with a cross-sectional design on 51 research subjects. This research was conducted at 5 Regional General Hospitals on the island of Lombok, namely the NTB Provincial Hospital, Mataram City Hospital, West Lombok District Hospital, Central Lombok District Hospital and East Lombok District Hospital from December 2021 - February 2022. Inclusion criteria were patients with benign and malignant thyroid nodules and normal thyroid function (T4 = 6-12 mg/dL; T3=0.2-0.3 mg/dL) while the exclusion criteria were patients not willing to participate in the study, thyroid nodules were not neoplasm, the patient has a tumor other than the thyroid.

Data Collection

Characteristics of subjects in the study according to inclusion and exclusion criteria based on age, sex, TIRADS, histological type, location and size of thyroid nodules <1 cm, 1.1-2 cm, 2.1-4 cm and >4 cm.

Assessment of TIRADS

TIRADS was determined in the evaluation of thyroid nodules using the USG GE logic S7 at the NTB Provincial Hospital, USG Versana Essential at the Mataram City Hospital, at the West Lombok District Hospital and at the Central Lombok District Hospital and USG GE P3 at the East Lombok District Hospital conducted by Radiologist. Ultrasound examination of the patient's thyroid was examined in the supine position with the neck hyperextended using a high frequency (7-15 MHz) linear transducer that provided adequate penetration and high image resolution. Scans are performed in the transverse and longitudinal planes. Real-time image of thyroid nodule is depicted using grayscale technique for TIRADS examination.

Assessment survivin expression

The patient's peripheral blood sample is taken from the patient's cubital vein. Nucleic acid extraction was carried out using a sample volume of about 100 ul of blood placed in 900 ul of a solution "L6" consisting of 120g of Guanidium thyocianate (GuSCN) (Fluka Chemie AG, Buchs, Switzerland, paint no. 50990) in 100 ml of 0.1 M Tris HCl, pH 6.4, 22 ml 0.2 M Ethylene Diamine Tetra Acetate (EDTA) pH 8.0 and 2.6g Triton X-100 (Packard, Instruments) with final concentrations 50 mM Tris HCl, 5 M GuSCN, 20 mM EDTA, 0.1 % Triton X -100. Then rotated at a speed of 12,000 rpm. The sediment was added to a 20 ul diatom suspension consisting of 50 ml H2O and 500 ul of 32% (w/v) "Celite" ("diatom") (Jansen Chimica, Beerse, Belgium, 10,846.79). Where 20 ul of this diatom suspension can bind 10 ug of blood RNA, then it is "vortexed" and centrifuged in a 1.5 ml eppendorf tube at a speed of 12,000 rpm for 15 minutes. The supernatant was removed and the sediment was washed with an "L2" solution consisting of 120 g of GuSCN in 100 ml 0.1 M Tris HCl, pH 6.4 by adding 1 ml of "L2" solution. Then it was vortexed and centrifuged at 12,000 rpm for 15 minutes, then the washing was repeated 2 times using "L2" solution, followed by washing with 1 ml of 70% ethanol 2 times and 1 ml of acetone. The results were then heated in a water bath at 56°C for 10 minutes and added 60 ul of "TE" solution consisting of 1 mM EDTA in 10 mM Tris HCL pH 8.0, then vortexed and continued by centrifugation at 12,000 rpm for 30 seconds, then incubated in oven for 10 minutes at 56°C. Then vortex and re-centrifuge for 30 seconds at a speed of 12,000 rpm and the supernatant was taken. The supernatant from this process will obtain nucleotide extraction results and store at -80°C before PCR analysis.

PCR was carried out on isolated DNA samples using the DNA amplification process. First, a PCR mix was made with a specific primer from survivin to be amplified containing 7.5 ml of qPCR mastermix from the reagent kit, then Forward primer was added. The primary nucleotide sequence of human survivin mRNA used was survivin Forward: 5′-CCCTGCCTGGCAGCCCTTTC-3′ (sense) and survivin Reverse: 5'-CTGGCTCCCAGCCTTCCA-3' (antisense), while housekeeping used Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) with the primary nucleotide sequence being GAPDH Forward: 5'-GGGAAACTGTGGCGTGAT-3' (sense) and GAPDH reverse: 5'-AAAGGTGGAGGAGTGGGT-3' (antisense). The PCR conditions were 35 cycles consisting of 1 minute of denaturation at 95°C and annealing for 20 seconds at 54°C. For relative quantification of gene expression, a calibration curve was made where GAPDH RNA, as a housekeeping enzyme, was used as an endogenous control. The calibration curve is as XY (scatter) and the plot represents the log of the input amount (log ng initial total mRNA) as the x-axis and Ct as the y-axis.

Histopathological examination

Histopathological examination using postoperative nodule tissue performed by a pathologist at each hospital.

Statistical Analysis

Data analysis used software SPSS version 22 and STATA 16 for windows. The test determined the comparison of the proportion of sex and age of the research sample. For the proportion of sex using the Chi-square test. Meanwhile, the Mann-Whitney test was used to compare the proportions of age. The comparison test of the mean and median predictor variables (TIRADS and survivin gene mRNA expression) on the histopathological results of thyroid nodules was then analyzed by bivariate test of the predictor variables on the histopathological results of benign and malignant nodules. Further data analysis used Receiver Operation Characteristic (ROC) and Area Under Curve (AUC) curves using STATA 16 software. From the ROC curve, the most optimal cut of sensitivity and specificity was searched for each predictor variable for histopathology of benign and malignant thyroid nodules. After finding the optimal cut-off point, the thyroid nodules are grouped based on the cut-off point. After that, it was processed using a 2x2 table. Then determined the value of sensitivity, specificity, accuracy,

Positive Predictive Value (PPV), Negative Predictive Value (NPV) of each variable as markers of benign and malignant thyroid nodules. Then the diagnostic test of a combination of predictor variables was carried out on the histopathological results of benign and malignant thyroid nodules.

RESULT

The characteristics of the subjects are dominated by female gender, age under 40 years old, benign histology, adenomatous goiter, right side location involvement, nodule size 1.1-2 cm, and the thyroid nodule was dominated by TIRADS 3 (Table 1 and 2). The relationship between TIRADS and histopathology and risk malignancy shows that the higher the TIRADS, the higher the risk of malignancy (see Table 3).

Analysis using Receiver Operator Characteristic (ROC) for each TIRADS, Survivin –mRNA gene expression using Stata-16 software. From the ROC TIRADS curve in determining thyroid nodules, it was found that the ability of TIRADS in diagnosing benign and malignant thyroid nodules was good with an area under curve (AUC) of 0.8064 (80.64%) (Figure 1).

From the ROC Survivin curve in determining thyroid nodules, it was found that the ability of Survivin in diagnosing benign and malignant thyroid nodules was excellent with an area under curve (AUC) of 0.9781 (97.81%) (Figure 2).

Combination screening test is a screening test that uses more than one examination test (multiple). There are two ways to use a combination screening test, the first serially (two-stage screening) and the second parallel (simultaneous screening). Each of these combination screening test methods has advantages and disadvantages. Each will also impact the results of the calculation of the net sensitivity and net specificity. For this reason, its use is adjusted to the needs or purposes of screening. If you want to get a multiple screening test that produces a higher sensitivity then it is done simultaneously or in parallel, but if you want a higher specificity then it is done in stages or serially. In the serial combination of TIRADS and survivin, there was an increase in diagnostic value, sensitivity 94.4%, specificity 75.8%, accuracy 82.35%, PPV 68%, NPV 96.2% (Table 4).

Table 1. Characteristic subject.

Characteristic	value
Sex	
Male	6 (11.76%)
Female	45 (88.24)
Age (year)	
≤40	31 (60.8%)
41-50	9 (17.6)
51-60	7 (13.7%)
>60	4 (7.8%)
TIRADS	
2	14 (27.5%)
3	27 (52.9%)
4	10 (19.61%)
Histology	
Benign	33 (64.71%)
Malignant	18 (35.29%)
Histology	
Adenomatous goiter	28 (54.9%)
Follicular adenoma	5 (9.8%)
Follicular thyroid carcinoma	1 (1.96%)
Papillary thyroid carcinoma	17 (33.33%)
Nodul Location	
Bilateral	7 (13.7%)
Right	27 (52.9%)
Left	17 (33.3%)
Nodule size (mean ± SD)	2.6 ± 0.173
Nodule size (cm)	
≤1	1(2%)
1.1-2	22 (43.1%)
2.1-4	20 (39.2%)
>4	8 (15.7%)

Table 2. Distribution of subject characteristics based on histopathological results.

Characteristics	Histopathology		total	p
	Benign (%)	Malignant (%)		
Sex				
Male	3 (9.1%)	3 (16.7%)	6 (11.8%)	0.625*
Female	30 (90.9%)	15 (83.3%)	45 (88.2%)	
Age (year)				
≤40	24 (72.7%)	7 (38.9%)	31 (60.8%)	0.037**
41-50	5 (15.2%)	4 (22.2%)	9 (17.6%)	
51-60	4 (12.1%)	3 (16.7%)	7 (13.7%)	
>60	0	4 (22.2%)	4 (7.8%)	

*Fisher's Exact Test; ** Mann-Whitney

DISCUSSION

Thyroid Imaging Reporting and Data System (TIRADS) is a scoring system developed for diagnosing and treating thyroid nodules in 2009. This system adopts the Breast Imaging Reporting and Data System (BIRADS) by the American College of Radiology (ACR) which is currently in use extensively on breast imaging. This system was introduced to overcome the problem of thyroid nodule selection that required fine

needle aspiration biopsy (FNAB) to be performed.[3] The TIRADS scoring system criteria continue to improve from time to time. In 2017, the American College of Radiology (ACR) released the latest criteria for the TIRADS scoring system.[4] After that in 2018 ACR also released "A user guide TIRADS 2017".[5]

Survivin is a protein consisting of 142 amino acids and has a molecular weight of 16.2 kDa. Meanwhile the size of the survivin gene is 15 kb. Survivin belongs to the Apoptotic Inhibitor (IAP) class and is the most potent inhibitor of apoptosis. In addition, survivin also has complex activities, namely accelerating cell transformation and playing a role in the process of mitosis and angiogenesis.[6] The role of survivin in the process of angiogenesis is demonstrated by the increased expression of survivin which protects endothelial cells from apoptosis in vitro.[7]

Tumor angiogenesis is highly dependent on the survival of the endothelium, survivin indirectly plays a role in protecting the new blood vessels that are formed. Survivin protein also works by suppressing the process of apoptosis and playing a role in cell proliferation. In addition, survivin is an IAP group with the smallest molecular size, expressed only in embryonal tissue and tumors.[8] Survivin genes also play a role in the differentiation, proliferation, infiltration and metastases of tumor cells. Moreover, survivin directly affects caspase signaling, especially in regulating caspase-3 and caspase-7 activity. It should be noted that caspase signaling is indirectly inhibited by survivin via P21.[6]

Survivin has activity to help in tumor development and metastasis. Survivin is a multi-functional protein that affects the regulation of mitosis, apoptosis, cellular mobility and can help tumor cell proliferation and metastasis. Survivin is also part of the Chromosomal Passenger Complex (CPC) along with aurora B kinase and Inner Centromere Protein (INCEP) to regulate chromosomal alignment during mitosis. Survivin also has interactions with Hepatitis B X-linked interacting protein (HBIXP) and X-linked IAP (XIAP) which can also prevent and regulate the activity of caspase-9 as a molecule involved in cell death. The anti-apoptotic effect of survivin can also be regulated by the pro-apoptotic SMAC molecule released from the mitochondria as a pathway of apoptosis. Survivin can accelerate cellular mobility through AKT activation and increase expression of 5 integrins.

Survivin plays a role in thyroid carcinoma. Evaluation of survivin expression in papillary and anaplastic carcinomas showed an increased expression in both. In several studies on thyroid nodules, an increase in immunohistochemical expression of survivin is associated with invasion, metastasis and tumor progression.[8] Increased immunohistochemical expression of survivin in medullary carcinoma is associated with increased blood calcitonin levels.

Research conducted by Tomas and Shah found that an increase in serum calcitonin levels was associated with the regulation of survivin.[7,9] Increased expression of survivin occurs during the

Table 3. TIRADS category with risk malignancy.

TIRADS category	Benign	Malignant	Total	Risk of Malignancy (%)
TIRADS 2	13 (39.4%)	1 (5.6%)	14 (27.5%)	7.1
TIRADS 3	19 (57.6%)	8 (44.4%)	27 (52.9%)	29.6
TIRADS 4	1 (3%)	9 (50%)	10 (19.6%)	90
Total	33 (64.7 %)	18 (35.3%)	51 (100%)	35.2

Table 4. Analysis of the cut-off value of the ROC TIRADS and Survivin.

	TIRADS	Survivin
AUC (95% CI)	80.64%	91.75%
Cut of point	3	7626
Sensitivity (95% CI)	94.4	100
Specificity (95% CI)	39.4	60.6
PPV (95% CI)	45.9	58.1
NPV (95% CI)	92.9	100
Accuracy (%)	58.82	74.5

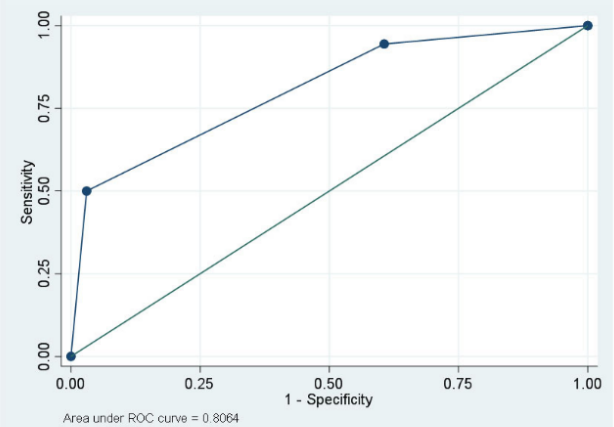

Figure 1. ROC TIRADS curve in determining thyroid nodules.

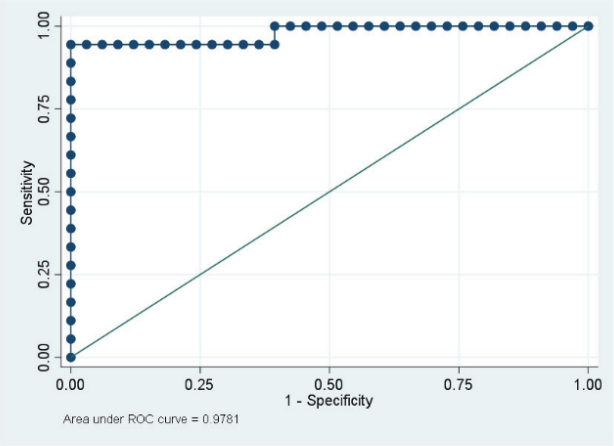

Figure 2. ROC Survivin curve in determining thyroid nodules.

occurrence of thyroid carcinoma. In this case, decreased and increased survivin expression is potentially useful as a marker for the diagnosis and prognosis of thyroid carcinoma.

Several studies on survivin expression were mostly carried out by tissue immunohistochemistry. To the researcher's knowledge, no studies have examined the expression of survivin in peripheral blood (circular tumor cells). So, further research is needed on the expression of survivin in thyroid carcinoma using peripheral blood samples (circular tumor cells).

CONCLUSION

The combination of thyroid ultrasound examination (TIRADS) and survivin gene mRNA expression using cubital vein blood samples can increase the diagnostic value of benign or malignant thyroid nodule types.

CONFLICT OF INTEREST

The researcher declares there is no conflict of interest in the research and publication.

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ETHICAL CONSIDERATION

This research has received ethical approval from the Health Research Ethics Committee, Faculty of Medicine, Universitas Hasanudin (Register Number: 442/UN4.6.4.5.31/PP36/2020).

AUTHOR CONTRIBUTION

All authors had contributed in manuscript writing and agreed for the final version of manuscript for publication.

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