International Journal of Endocrinology

Оригінальні дослідження
/Original Researches/

UDC 618.19-006.6-078.33-091.8 DOI: https://doi.org/10.22141/2224-0721.17.3.2021.232652

Gulhan Duman, Baris Sariakcali
Division of Endocrinology and Metabolism, Department of Internal Medicine, Cumhuriyet University, Faculty of Medicine, Sivas, Turkey

Serum WNT-induced secreted protein 1 level as a potential biomarker for thyroid nodules

For citation: Mìžnarodnij endokrinologìčnij žurnal. 2021;17(3):226-233. doi: 10.22141/2224-0721.17.3.2021.232652

Abstract. ***Background.*** *Thyroid nodule (TN) is a common thyroid disease worldwide, and it has increased significantly last decades. Most TNs are usually incidental findings of asymptomatic, benign lesions discovered by imaging modalities performed for reasons unrelated to thyroid diseases. The purpose of this study was to investigate the value of serum WNT-induced secreted protein 1 (WISP1) level as a supporting biomarker to perform differential diagnosis of benign and non-benign thyroid nodules.* ***Materials and methods.*** *The study was completed with the 89 patients undergone fine needle aspiration biopsy and 43 controls. The patients were composed of 96 (72.7 %) females and 36 (27.3 %) males. And they were divided into 2 group according to the Bethesda cytological evaluation as Benign (Bethesda 2) and Non-Benign (Bethesda 3–6) groups. Their serum WISP1 levels were measured by an ELISA method.* ***Results.*** *There were 58 (43.9 %) patients in Benign (Bethesda 2) and 31 (23.5 %) in non-Benign (Bethesda 3–6) groups. In the contrary nodule size was bigger in the Non-benign group than that benign group (p = 0.006). The serum WISP1 level in the Benign (Bethesda 2) group was significantly higher than that in the and Non-Benign (Bethesda 3–6) group, and controls (p < 0). The difference between benign and non-benign group accordingly to their echogenicitiy was significant (p < 0.05). In benign group there was 76.9 % mixed echoic nodules, 76.7 % isoechoic nodules 68.4 % isohypoechoic nodules and 35.7 % hypoechoic nodules. In the non-benign group, the highest hypoechoic echo (64.3 %), the least mixed echo (23.1 %), while in the benign group, the most mixed echo (76.9 %), the least hypoechoic echo (35.7 %) was present. There was no relation between WISP1 levels and echogenicity with Kruskal-Wallis H test.* ***Conclusions.*** *According to the preliminary results of current study, addition of serum WISP1 measurement to the differential diagnostic work-up of thyroid nodules patients may provide supportive information. In thyroid nodules patients with Benign (Bethesda 2) category of cytological evaluation, a higher level of serum WISP1 may support cytological diagnosis.*

Keywords: *thyroid nodule; thyroid ultrasonography; fine-needle aspiration biopsy; WISP1*

Introduction

Thyroid nodule (TN) is a common thyroid disease worldwide, and it has increased significantly last decades [1]. Most TNs are usually incidental findings of asymptomatic, benign lesions discovered by imaging modalities performed for reasons unrelated to thyroid diseases. Thyroid nodules are detected clinically in 5 % of females and in 1 % of males in non-endemic areas. Thyroid malignancy can be detected in up to 15 % of these nodules due to gaining malignancy [2, 3] therefore, the thyroid surgeon is dependent on diagnostic studies to decide when surgery is necessary [4].

In order to further evaluate these nodules, fine-needle aspiration (FNA) is widely accepted as the primary diagnostic tool for the evaluation of TNs owing to its simplicity, safety, and cost-effectiveness [5]. FNA biopsy (FNAB) remains to be mandatory and valuable method used in the

For correspondence: Dr. Gulhan Duman, Division Endocrinology, Department of Internal Medicine, Sivas Cumhuriyet University, Faculty of Medicine, 58140, Sivas, Turkey; e-mail: gulcavlak@hotmail.com, contact phone: 0 (346) 2191010; fax: 0 (346) 2580000.
Full list of authors information is available at the end of the article.

evaluation of TNs, but it is still not sufficient as a standard process. Many studies have shown high rate of FNAB specificity (86–100 %) and sensitivity (93–100 %), as well as low rate of false-negative results (3–6 %) [2, 6]. The quality and quantity FNAB applied has been maximized according to the Bethesda cytological evaluation system. A preoperative FNAB should provide all clinical data that can shape the best and appropriate individual treatment for each patient. Yet, the precisely differentiating benign and malignant nodules is crucial and directly related to an accurate management [2, 7].

With technological advancements, diagnostic capabilities of ultrasonography (US) are considerable increased during diagnostic and follow-up procedures as well as guidance during FNAB. Despite the wide application of FNA, the diagnostic yield is limited to 80 to 99 %. Undetermined cytological results always result in confusion and the use of repeated FNA after a non-diagnostic result is still questionable [8, 9]. There is a need new diagnostic tools to reduce unsatisfactory diagnostic results. Within this context, there is a need to the development of a clinically meaningful diagnostic biomarkers to obtain additional clinical data useful for differential diagnosis of TNs without higher cost and increased risk for patients.

Various molecular tests are used to determine whether patients with thyroid nodules that result in indeterminate FNA have cancer. B-Raf proto-oncogene (BRAF), Rat sarcoma viral (RAS) include KRAS, HRAS, NRAS point mutations and ret proto-oncogene (RET/PTC), peroxisome proliferator-activated receptor gamma (PAX8/PPARγ) rearrangements are the most commonly used molecular panels for this purpose. These molecular tests increase the diagnostic power of FNA and thus help to learn more about the biological behavior of the tumor preoperatively [10]. However, no relationship was found between BRAF-V600E KRAS, NRAS mutations in the study conducted by O.İ. Özdamar et al. in thyroid cancer patients with Hashimoto's disease [11].

Because molecular tests are not easy to access, expensive and patients are not willing to use an invasive method such as biopsy, the search for biomarkers that can be easily detected in serum has recently become popular. For this purpose, many biomarkers such as circulating miRNAs, platelets, serum calprotectin, matrix metalloproteinase (MMP), Midkine pleiotropic growth factor, vascular adhesion protein 1 (VAP-1), galectin-3 and interleukins (IL-6, 8, 10) etc. have been investigated to distinguish benign and malign nodules [12].

Up to now, there is no reliable biomarker used to get information about nature of TNs. Among the potential signal transduction growth factors, Wnt inducible signaling pathway protein 1 (WISP1), defined also Cellular Communication Network (CCN4), is a member of the connective tissue growth factor/cysteine-rich 61/nephroblastoma overexpressed family. It is one of the secreted matricellular proteins in the extracellular matrix and has many cellular functions in a highly tissue-specific manner, from cell survival to proliferation. Interestingly, elevated WISP1 expression has also been observed in a variety of cancers such as gastric, colon, lung, liver, and breast cancers and melanoma further confound the role of WISP1 in carcinogenesis [13–15].

We hypothesized that WISP1 could be involved in the development of thyroid tumors and investigated the value of serum WISP1 in patients undergone FNAB followed by Bethesda cytological evaluation. As far as we know, no previous study has evaluated the association of serum WISP1 value with Bethesda cytology criteria of TN patients. The objective of this study was to search the value of serum WISP1 level as a supporting biomarker to perform diagnostic work-up of benign and malignant TNs.

Materials and Methods

Patients

The Human Research Ethics Committee of our institution (Registry No: 2019-02/06) approved this study. All patients provided their informed consent before diagnostic procedures. Ultrasound guided FNAB was conducted according to relevant guidelines and regulations. Fine-needle aspiration biopsy accompanied by US was applied to eligible patients with normal TSH who applied to our department for the evaluation of thyroid nodules between January 2019 and July 2019. The patients with diabetes mellitus, obesity (body mass index ≥ 30), chronic kidney disease, chronic heart disease, chronic liver disease, rheumatological disease, cancer and smoking were excluded from the study. The control group included people who applied to find out if they had nodules, but no nodules were detected. The control group also did not have any chronic diseases or habits mentioned above.

Briefly, FNAB was performed using a 27-gauge needle and a 20-ml syringe under US guidance for all nodules bigger than 1 cm and nodules smaller than or equal to 1 cm with at least one suspicious US finding. FNAB samples were arranged as smears after air-dried.

Cytological evaluation of FNAB specimens was conducted in accordance with the Bethesda System for Reporting Thyroid Cytopathology in 2008 [7]. There are six group according to cytological classification: Bethesda 1, non-diagnostic; Bethesda 2, Benign; Bethesda 3, Atypical of undetermined significance/follicular lesions of undetermined significance; Bethesda 4, Follicular neoplasm/suspicious for follicular neoplasm; Bethesda 5, Suspicious for malignancy; and Bethesda 6, Malignant [16]. In this study, cytology data were presented in the following subsets: Benign (Bethesda 2), non-benign (Bethesda 3–6).

Measurement of WISP1

After overnight fasting, blood samples were collected from all participants into red top tubes (Becton Dickinson, Oxford, UK). Samples were taken on the day of admission to the hospital. The serum sample tubes were allowed to clot before centrifugation. After centrifugation at 4 °C for 15 min at 3.500 rpm, the serum was aliquoted and immediately frozen at –40 °C.

Serum WISP1 levels measured by Abbkine ELISA kit (China). According to the ELISA kit procedure, the fol-

lowing operations were carried out respectively: standard working solutions were prepared in 5 Eppendorf tubes with concentrations of 480, 240, 120, 60, 30, 15 picograms per milliliter (pg/mL), respectively. 50 µL standards were added to the ELISA plate and 40 µL sample diluent was added to the remaining parts. Ten µl samples were added to the wells with sample diluents. Plate was covered and incubated at 37 °C for 45 min. Plate was washed 5 times with washing solution. Then 50 µL of HRP-conjugate was added to all wells and then it was placed at 37 °C for 30 min. Plate was washed 5 times with washing solution and 50 µl chromogen solution A and 50 µL chromogen solution B was added. It was incubated for 15 min at 37 °C, protected from light. 50 µL of stop solution was added to each well. Absorbance values were read at 450 nm. Concentrations of samples were calculated according to the absorbance values of the standards, the amount of WISP1 was calculated in pg/mL.

Statistical analysis

SPSS 25.0 (IBM SPSS Statistics for Windows, Version 25.0. Armonk, NY: IBM Corp.) package statistics program was used to analyze the data obtained in the study. Before the analysis and evaluations, the compatibility of the obtained data with the normal distribution was tested by performing Histogram and One Sample Kolmogorow-Smirnow test, and it was observed that the data did not have a normal distribution feature. For this reason, besides the frequency distributions, non-parametric tests (Chi-Square, Mann-Whitney U test and Kruskal-Wallis tests) were used. ROC analysis was used to determine the cut-off point to predict benign and non-benign nodules with WISP1 levels. A p value of < 0.05 accepted as statistically significant.

Results

The study was completed with the 89 patients undergone FNAB and 43 controls. The patients were composed of 96 (72.7 %) females and 36 (27.3 %) males. The controls consisted of 28 (65.1 %) females and 15 (34.9 %) males. We divided the patient Benign (Bethesda 2) and Non-Benign (Bethesda 3–6) groups according to the FNAB results. Benign group consisted of 58 (43.9 %) and non-benign group consisted of 31 (23.5 %) patients. There was no difference in terms of age and sex among Benign, non-Benign and Control groups. Selected demographic characteristics of patients undergone FNAB were presented in table 1.

According toWISP1 levels, there is statistically significant difference between benign and non-benign groups. Mean WISP1 level in Benign group is 302.165 ± 88.760, 197.295 ± 48.970 pg/mL in non-Benign group and 234.12 ± 41.39 pg/mL in control group. In the Benign group, the mean level of WISP1 level in women is 286.40 ± 143.73 and 317.93 ± 33.79 pg/mL in men. In the non-Benign

Table 1. Demographic characteristic of the participants

Parameters			Group			χ^2	sd	*p*
			Control	Benign	Non-Benign			
Sex	Female	n	28	46	22	2.571	2	0.276
		%	29.2	47.9	22.9			
	Male	n	15	12	9			
		%	41.7	33.3	25.0			
Age	20–35	n	9	6	6	5.838	6	0.442
		%	42.9	28.6	28.6			
	36–50	n	17	21	7			
		%	37.8	46.7	15.6			
	51–65	n	9	19	12			
		%	22.5	47.5	30.0			
	66+	n	8	12	6			
		%	30.8	46.2	23.1			

Table 2. WISP1 levels according benign and non-benign groups

Sex	Variable	n	Mean	Mean Rank	SS	KW	*p*
Female	Control	28	234.12	41.93	173.22	12.931	0.002*
	Benign	46	286.40	58.86	143.73		
	Non-Benign	22	185.70	35.20	743.69		
Male	Control	15	274.05	16.7	52.61		
	Benign	12	317.93	23.88	33.79	5.014	0.082
	Non-Benign	9	208.00	14.22	18.67		

Note: * — p < 0.05.

group, the average level of WISP1 is 185.81 ± 76.19 in women and 208.78 ± 21.15 pg/mL in men While WISP1 level is higher in benign group, it's lower in non-benign group (p = 0). This significant difference between benign and non-benign groups is related to the higher WISP1 level in women. In addition, in the ROC analysis, WISP1 cutoff value was found to be 231.24 pg/mL with 73 % sensitivity, 63 % sensitivity and 69 % accuracy respectively. Patients with WISP1 levels above 231.24 pg/mL are more likely to be benign than non-benign. Although WISP1 levels were higher in men in group benign, it was not statistically significant. Mann Whitney U test results used to analyze whether the test results of the patients participating in the study show a significant difference according to the WISP1 levels are given in table 2. Figure 1 depicts the serum WISP1 levels of patients with TNs categorized as Benign (Bethesda 2) and Non-Benign (Bethesda 3–6) groups and controls.

There is a statistically significant difference in Thyroid Stimulating hormone (TSH) levels in patients Benign and non-Benign groups (p < 0.05). Mean TSH level in non-Benign group is 2.5 IU/μIU/mL and 1.9 in Benign group. Accordingly, while TSH level is significantly lower in benign group than in non-benign group (p = 0.048) (table 3).

There is significant difference in nodule size and nodule count between benign and non-benign groups (p < 0.006). The average nodule size in Non–Benign group is 22.92 ± 16.74 mm and 18.57 ± 7.60 mm in Benign group. The size of nodule significantly smaller in benign group than the non-benign one. Similarly, the count of nodules also shows a statistically significant difference between benign and non-benign groups (p < 0.006). While nodule numbers in Benign group is 2.64 ± 1.77, it is 1.92 ± 1.95 in Non-Benign group. The nodule number is higher in benign group than in the non-benign group (table 4). There is no relationship between nodule number and nodule size with WISP1 levels in Sperman Rho test.

The difference between benign and non-benign group accordingly to their echogenicitiy was significant (p < 0.05). In benign group there was 76.9 % mixed echoic nodules, 76.7 % isoechoic nodules 68.4 % isohypoechoic nodules and 35.7 % hypoechoic nodules. In the non-benign group, the highest hypoechoic echo (64.3 %), the least mixed echo (23.1 %), while in the benign group, the most mixed echo (76.9 %), the least hypoechoic echo (35.7 %) was present (table 5). There was no relation between WISP1 levels and echogenicity with Kruskal-Wallis H test.

Table 3. TSH levels in Benign and Non-Benign Groups

Variable		Mean	Mean rank	SS	Z	*p*
TSH Levels	Non-benign	2.50	46.88	1.51	–1.976	0.048
	Benign	1.91	36.02	165		

Note: Whether the variables show the first distribution or not has been tested; Skewness values were not found between 1.5 and +1.5 (–1.53/0.16) and it was accepted that there was no first distribution (Tabachnick and Fidell, 2013).

Table 4. Nodule Size and Nodule Count in Benign and Non-Benign Nodules

Variable		n	Mean	Mean rank	SS	Z	*p*
Nodule Size	Non-benign	25	22.92	39.44	16.74	–2.769	0.006
	Benign	53	18.57	39.53	7.60		
Nodule Count	Non-benign	24	1.92	28.96	1.95	–2.769	0.006
	Benign	53	2.64	43.55	1.77		

Table 5. The Relationship Echogenicity with Benign and Non-Benign Groups

Parametres			Groups		Total
			Non-benign	Benign	
Echogenicity	Mixed echoic	n	3	10	13
		%	23.1	76.9	100.0
	Isoechoic	n	7	23	30
		%	23.3	76.7	100.0
	Isohypoechoic	n	6	13	19
		%	31.6	68.4	100.0
	Hypoechoic	n	9	5	14
		%	64.3	35.7	100.0
Total		n	25	51	76
		%	32.9	67.1	100.0

Notes: p = 0.044, χ^2 = 8.075, df: 3.

Discussion

To the best of our knowledge, current study is the first evaluation of diagnostic value of serum WISP1 in TN patients. The study was completed with the 89 patients undergone FNAB and 43 controls. We divided the patient and groups according to the FNAB results. The patients with Benign (Bethesda 2) category possessed meaningfully higher levels of serum WISP1 compared to that of the controls and Non-Benign (Bethesda 3–6) patients.

During diagnostic work-up of TNs, with US examination, to determine nature of the TN as benign or malign is not easy in many clinical conditions before decision to surgical intervention. Need for surgical intervention of TN is considerably decreased in last decades after successfully performed FNAB; however, yet it is not clear how to manage long-term follow-up of these patients. One of the important concerns during this follow-up is the possibility of benign to malignant transformation that is a subject of research recently [17]. For shedding light on this subject, Arora et al. conducted a retrospective study investigating the status benign to malignant transformation of thyroid tumors [17]. They noted that the review of our their 10-year experience with thyroid tumors including also screening of some cytological and molecular tumor markers revealed that the rate benign to malignant transformation of thyroid tumors was 2 % in their series.

Generally, FNAB is recommended for TNs of 10 mm or larger in diameter, presenting with a high- or intermediate-suspicion pattern on ultrasonography; TNs 15 mm or larger in diameter, showing a low-suspicion pattern on ultrasonography; and TNs 20 mm or larger in diameter, presenting with a very-low-suspicion pattern on ultrasonography. During first evaluation, TNs considered to be suspicious by medical history or ultrasonographic characteristics should be considered for FNAB when their size is less than 10 mm in diameter [5, 18].

For the appropriate management of patients with TNs, US and FNAB are accepted as the most useful diagnostic modalities. The possibility of malignancy increases significantly with the higher Bethesda rating [19]. Hence, it is useful for the clinicians to be able to predict the possibility of the cytology results. The large-scale use of FNAB in TN patients during surgical evaluation has resulted in an increase in malignant types of specimens as revealed after thyroidectomies by more than 50 %, and additionally, the number of TN patient's undergone surgery has decreased by 50 % [2].

An identification of malignancy in TNs can reduce missed diagnosis and delayed management, avoiding also surgery for benign TNs, thus decreasing the physical and psychological burden of malignancy. Within this context, since the features of atypical benign and malignant TNs may overlap on routine US [20]. We need new diagnostic modalities to improve our diagnostic capabilities. A relationship with malignancy was found if the level of midline pleotropic growth factor used to distinguish benign and malignant nodules was higher than 323.12 pg/mL, its sensitivity, specificity, and diagnostic accuracy rates of 75.70, 75.00, and 75.31 %, respectively [21]. In another study performed

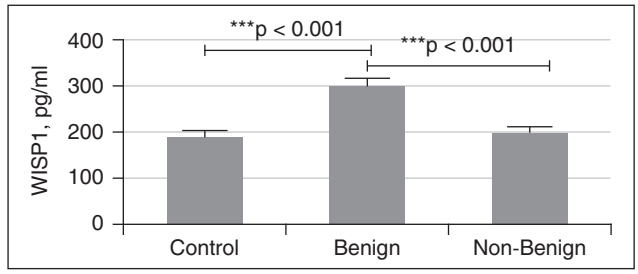

Figure 1. Serum WISP1 levels of patients with TNs categorized as Benign, Non-Benign (Bethesda 3–6) and controls. Serum WISP1 level of patients in Benign group was significantly higher than that in the Non-Benign and control groups (p < 0.001)

with vascular adhesion protein-1 (VAP-1), the cutoff was found to be 456.6 ng/mL and it was found that the VAP-1 level was lower in patients with thyroid cancer than in healthy individuals without benign nodules with a specificity of 77.4 % and a sensitivity of 66.7 % respectively [22].

Similar to the VAP-1 study mentioned above, we found that WISP1 levels were significantly lower in the non-benign group compared to the benign group in our study. Patients with WISP1 levels above 231.24 pg/mL are more likely to be benign than non-benign with 73 % sensitivity, 63 % sensitivity and 69 % accuracy respectively. Therefore, we speculated that if the basal levels of WISP1 levels are known in the benign and non-benign groups, the change of these levels in the future may be a sign of malignant transformation.

It is clear, that evaluation of the thyroid FNABs with the Bethesda cytological evaluation system increases the reliability of cytological diagnosis. However, the impact of Bethesda application may vary among different institutions. Clinicians need to consider the malignancy rate in the Bethesda categories in their hospitals to increase the accuracy of their investigation and decision in the TN patients [23].

According to the results of the study of D.A. Kleiman et al. the authors suggested that decisions about need of FNAB may be based on the presence of suspicious US findings in order to exclude or confirm malignancy [24]. They found that US findings that were significantly associated with malignant diagnosis were microcalcifications, irregular shape, irregular margins and hypoechogenicity of a nodule. They noted that even for smaller nodules of 10 mm with high suspicious pattern, FNAB need to be considered [24]. The US features of TNs in the patients in our study was found somewhat in accordance with those results, although the numbers of patients with these features did not allowed the performance of statistical comparisons.

If the FNAB contains at least six groups that produce ten preserved follicular epithelium cells, accepted as sufficient [7]. Bethesda category 1 cytology results from inadequate sample collection with FNAB and is about 1.8–23.6 % of FNAB results. In many studies, FNAB has shown its sensitivity and specificity to prevent unnecessary surgical procedures, but the technique and the nature of the lesion are very important for an adequate sample. Adequate sample aspiration from sclerotic, calcific and cystic lesions is not an easy procedure [6, 16].

We think that adding a biomarker such as serum WISP1 giving information about benign or malign nature of TNs may be helpful to reach a decision about a second FNAB.

WISP1 gene expression has been shown to be rearranged in diseases such as cancer, diabetic nephropathy, retinopathy and fibrotic diseases. WISP1 is stimulated by the pathway of WINT-1 and B-catenin thus contributes to tumorigenesis [25, 26]. Many studies have demonstrated that WISP1 is related to different kind of tumor development. WISP1 performs its effect using many signal paths, some of these are those mitogen-activated protein kinase (MAPK), protein kinase B (Akt), phosphatidylinositol 3-kinase (PI3K), Jun N-terminal kinase (JNK), caspases, forkhead transcription factors, sirtuins, c-myc oncogene, glycogen synthase kinase 3β (GSK-3β), β-catenin, mammalian target of rapamycin (mTOR) and microRNAs (miRNAs). Through these signal pathways, WISP1 can affect and change the route of cell death programs such as autophagy and apoptosis to protect cytoprotection and tissue repair [27].

Excess gene expression of WISP1 regulates cell adhesion of colon cancer and contributes to poor prognosis. WISP1 worsens the course of oral squamous cell carcinoma through angiogenesis. In esophageal squamous cell carcinoma, WISP1 elevation plays a role as a marker of poor prognosis and a regulator of tumor cell radio-sensitivity. These studies vigorously suggest that WISP1 plays an important role in tumor development of various tumors and might be use a target molecule of tumor therapy [28]. WISP1 coordinates cell proliferation, apoptosis, migration, invasion, and chemotherapeutic resistance of glioblastoma [29].

High levels of WISP1 are associated with poor prognosis in breast, esophageal and rectal cancers, while in melanoma predicts poor outcome at low WISP1 levels [30–33]. In addition, L.L. Soon et al. demonstrated that overexpression of WISP1 in lung cancer inhibits metastasis, in vitro invasion, and motility through Rac regulatory activation [34].

The mouse fibroblast which have over-expressed WISP1, could not form colonies on the soft agar, but when it was injected into the mouse it formed tumors. This suggested that positive paracrine interaction is necessary for tumor development and progression [21]. On the contrary, the fact that H. Shao et al. shown WISP1 is lower in the melanoma cells and fibroblasts activated by melanoma than the fibroblasts in the surrounding tissue suggested the negative paracrine regulatory effect [33]. The crosstalk between tumor cells and the tumor stroma has a considerable effect on tumor progression. Tumor release some growth factors and proteases that will stimulate stromal cells in the surrounding tissues to create a suitable environment for tumor cell progression [35]. This activated carcinoma-associated fibroblasts (CAFs), secrete extracellular matrix proteins and matrix degrading enzymes to regulate microenvironment [35].

In colon cancer and in breast cancer WISP1 is upregulated in CAFs more than in fibroblasts at the adjacent normal tissue [36, 37]. In many cancers WISP1 is accumulated in the tumor stroma which around the cancer cells [22, 36–39]. In an experimental animal study, it has been shown that serum and tissue WISP1 levels are high in the early stages of prostate cancer, and the severity of the disease increases with the decrease in tissue and serum WISP1 levels. These studies suggested that WISP1 can be used as a tumor marker such as Prostate Specific Antigen (PSA) [33].

As a result, WISP1 is a dual-acting molecule with an oncogenic effect in some tumors and suppressor in others [40]. Low levels in melanoma increased tumor progression [33], while high levels in lung and prostate cancer suppressed invasion and metastasis [40]. Similar to these cancers, low WISP1 levels were found to be associated with non-benignity in our study.

Although this study has some limitations, whether WISP1, which is used as a follow-up parameter in some malignant cancers, can be used in the follow-up of benign TNs needs to be considered. For this purpose, larger-scale prospective studies are needed to suggest that WISP1 can be used as a diagnostic tool for identifying TNs as benign or malignant. Since recently have revealed a stable and marked rise in the occurrence of thyroid cancer worldwide, the need for biomarkers that may be useful for primary diagnosis and follow-up of TNs in addition to the skilled aspiration, skilled cytological interpretation and rational analysis of cytological and clinical data.

Conclusions

Nowadays, transformation mechanism from benign nodule to malignant nodule and affecting factors remain elusive. WISP1 may serve as a potential molecular biomarker for TNs. According to the preliminary results of current study, addition of serum WISP1 measurement to the differential diagnostic work-up of TN patients may provide supportive information. In TN patients with Bethesda 2 category of cytological evaluation, a higher level of serum WISP1 may support cytological diagnosis and long-term follow up. In TN patients with other Bethesda categories of cytological evaluation, a low level of serum WISP1 may support cytological diagnosis. Further studies need to be performed to determine the predictive value of serum WISP1 as a candidate biomarker to differentiate benign and malign TNs according to their final diagnosis after surgery.

Acknowledgement. We would like to thank Assoc. Prof. Halef Okan Dogan for running and evaluating WISP1 Elisa kits.

References

1. Al Dawish MA, Robert AA, Muna A, et al. Bethesda System for Reporting Thyroid Cytopathology: A three-year study at a tertiary care referral center in Saudi Arabia. World J Clin Oncol. 2017 Apr 10;8(2):151-157. doi:10.5306/wjco.v8.i2.151.

2. Janczak D, Pawlowski W, Dorobisz T, et al. An evaluation of the diagnostic efficacy of fine needle aspiration biopsy in patients operated for a thyroid nodular goiter. Onco Targets Ther. 2016 Sep 22;9:5819-5823. doi:10.2147/OTT.S111275.

3. Dean DS, Gharib H. Epidemiology of thyroid nodules. Best Pract Res Clin Endocrinol Metab. 2008 Dec;22(6):901-911.

doi:10.1016/j.beem.2008.09.019.

4. Bomeli SR, LeBeau SO, Ferris RL. Evaluation of a thyroid nodule. Otolaryngol Clin North Am. 2010 Apr;43(2):229-238, vii. doi:10.1016/j.otc.2010.01.002.

5. Haugen BR, Alexander EK, Bible KC, et al. 2015 American Thyroid Association Management Guidelines for Adult Patients with Thyroid Nodules and Differentiated Thyroid Cancer: The American Thyroid Association Guidelines Task Force on Thyroid Nodules and Differentiated Thyroid Cancer. Thyroid. 2016 Jan;26(1):1-133. doi:10.1089/thy.2015.0020.

6. Yang J, Schnadig V, Logrono R, Wasserman PG. Fine-needle aspiration of thyroid nodules: a study of 4703 patients with histologic and clinical correlations. Cancer. 2007 Oct 25;111(5):306-315. doi:10.1002/cncr.22955.

7. Baloch ZW, LiVolsi VA, Asa SL, et al. Diagnostic terminology and morphologic criteria for cytologic diagnosis of thyroid lesions: a synopsis of the National Cancer Institute Thyroid Fine-Needle Aspiration State of the Science Conference. Diagn Cytopathol. 2008 Jun;36(6):425-437. doi:10.1002/dc.20830.

8. Meko JB, Norton JA. Large cystic/solid thyroid nodules: a potential false-negative fine-needle aspiration. Surgery. 1995 Dec;118(6):996-1003; discussion 1003-4. doi:10.1016/s0039-6060(05)80105-9.

9. Shin JJ, Caragacianu D, Randolph GW. Impact of thyroid nodule size on prevalence and post-test probability of malignancy: a systematic review. Laryngoscope. 2015 Jan;125(1):263-272. doi:10.1002/lary.24784.

10. Hsiao SJ, Nikiforov YE. Molecular approaches to thyroid cancer diagnosis. Endocr Relat Cancer. 2014 Oct;21(5):T301-313. doi:10.1530/ERC-14-0166.

11. Özdamar Oİ, Acar GÖ, Özen F, Zenginkinet T. Assessment of BRAF V600E, KRAS, NRAS and EGFR mutations in papillary thyroid carcinoma and Hashimoto thyroiditis. ENT Updates. 2020;10(2):300-305. doi:10.32448/entupdates.711666.

12. Wang W, Chang J, Jia B, Liu J. The blood biomarkers of thyroid cancer. Cancer Manag Res. 2020 Jul 6;12:5431-5438. doi:10.2147/CMAR.S261170.

13. Chiang KC, Yeh CN, Chung LC, et al. WNT-1 inducible signaling pathway protein-1 enhances growth and tumorigenesis in human breast cancer. Sci Rep. 2015 Mar 3;5:8686. doi:10.1038/srep08686.

14. Deng W, Fernandez A, McLaughlin SL, Klinke DJ 2nd. WNT1-inducible signaling pathway protein 1 (WISP1/CCN4) stimulates melanoma invasion and metastasis by promoting the epithelial-mesenchymal transition. J Biol Chem. 2019 Apr 5;294(14):5261-5280. doi:10.1074/jbc.RA118.006122.

15. Jia S, Qu T, Feng M, et al. Association of Wnt1-inducible signaling pathway protein-1 with the proliferation, migration and invasion in gastric cancer cells. Tumour Biol. 2017;39(6):1010428317699755. doi:10.1177/1010428317699755.

16. Evranos B, Polat SB, Baser H, et al. Bethesda classification is a valuable guide for fine needle aspiration reports and highly predictive especially for diagnosing aggressive variants of papillary thyroid carcinoma. Cytopathology. 2017 Aug;28(4):259-267. doi:10.1111/cyt.12384.

17. Arora N, Scognamiglio T, Zhu B, Fahey TJ 3rd. Do benign thyroid nodules have malignant potential? An evidence-based review. World J Surg. 2008 Jul;32(7):1237-1246. doi:10.1007/s00268-008-9484-1.

18. Russ G, Bonnema SJ, Erdogan MF, Durante C, Ngu R, Leenhardt L. European Thyroid Association Guidelines for Ultrasound Malignancy Risk Stratification of Thyroid Nodules in Adults: The EU-TIRADS. Eur Thyroid J. 2017 Sep;6(5):225-237. doi:10.1159/000478927.

19. Bongiovanni M, Spitale A, Faquin WC, Mazzucchelli L, Baloch ZW. The Bethesda System for Reporting Thyroid Cytopathology: a meta-analysis. Acta Cytol. 2012;56(4):333-339. doi:10.1159/000339959.

20. Ha EJ, Baek JH, Na DG. Risk stratification of thyroid nodules on ultrasonography: current status and perspectives. Thyroid. 2017 Dec;27(12):1463-1468. doi:10.1089/thy.2016.0654.

21. Meng Z, Tan J, Zhang G, et al. Evaluation of serum midkine as a biomarker in differentiated thyroid cancer. Life Sci. 2015 Jun 1;130:18-24. doi:10.1016/j.lfs.2015.02.028.

22. Hu Z, Zhao P, Zhang K, Zang L, Liao H, Ma W. Evaluation of Serum Vascular Adhesion Protein-1 as a Potential Biomarker in Thyroid Cancer. Int J Endocrinol. 2016;2016:6312529. doi:10.1155/2016/6312529.

23. Cibas ES, Ali SZ. The 2017 Bethesda System for Reporting Thyroid Cytopathology. Thyroid. 2017 Nov;27(11):1341-1346. doi:10.1089/thy.2017.0500.

24. Kleiman DA, Beninato T, Soni A, Shou Y, Zarnegar R, Fahey TJ 3rd. Does bethesda category predict aggressive features in malignant thyroid nodules? Ann Surg Oncol. 2013 Oct;20(11):3484-3490. doi:10.1245/s10434-013-3076-5.

25. Pennica D, Swanson TA, Welsh JW, et al. WISP genes are members of the connective tissue growth factor family that are up-regulated in wnt-1-transformed cells and aberrantly expressed in human colon tumors. Proc Natl Acad Sci U S A. 1998 Dec 8;95(25):14717-14722. doi:10.1073/pnas.95.25.14717.

26. Xu L, Corcoran RB, Welsh JW, Pennica D, Levine AJ. WISP-1 is a Wnt-1- and beta-catenin-responsive oncogene. Genes Dev. 2000 Mar 1;14(5):585-595.

27. Maiese K. WISP1: Clinical insights for a proliferative and restorative member of the CCN family. Curr Neurovasc Res. 2014;11(4):378-389. doi:10.2174/1567202611666140912115107.

28. Gurbuz I, Chiquet-Ehrismann R. CCN4/WISP1 (WNT1 inducible signaling pathway protein 1): a focus on its role in cancer. Int J Biochem Cell Biol. 2015 May;62:142-146. doi:10.1016/j.biocel.2015.03.007.

29. Wu J, Long Z, Cai H, et al. High expression of WISP1 in colon cancer is associated with apoptosis, invasion and poor prognosis. Oncotarget. 2016 Aug 2;7(31):49834-49847. doi:10.18632/oncotarget.10486.

30. Taghavi A, Akbari ME, Hashemi-Bahremani M, et al. Gene expression profiling of the 8q22-24 position in human breast cancer: TSPYL5, MTDH, ATAD2 and CCNE2 genes are implicated in oncogenesis, while WISP1 and EXT1 genes may predict a risk of metastasis. Oncol Lett. 2016 Nov;12(5):3845-3855. doi:10.3892/ol.2016.5218.

31. Nagai Y, Watanabe M, Ishikawa S, et al. Clinical significance of Wnt-induced secreted protein-1 (WISP-1/CCN4) in esophageal squamous cell carcinoma. Anticancer Res. 2011 Mar;31(3):991-997.

32. Davies SR, Davies ML, Sanders A, Parr C, Torkington J, Jiang WG. Differential expression of the CCN family member

WISP-1, WISP-2 and WISP-3 in human colorectal cancer and the prognostic implications. Int J Oncol. 2010 May;36(5):1129-1136. doi:10.3892/ijo_00000595.

33. Shao H, Cai L, Grichnik JM, Livingstone AS, Velazquez OC, Liu ZJ. Activation of Notch1 signaling in stromal fibroblasts inhibits melanoma growth by upregulating WISP-1. Oncogene. 2011 Oct 20;30(42):4316-4326. doi:10.1038/onc.2011.142.

34. Soon LL, Yie TA, Shvarts A, Levine AJ, Su F, Tchou-Wong KM. Overexpression of WISP-1 down-regulated motility and invasion of lung cancer cells through inhibition of Rac activation. J Biol Chem. 2003 Mar 28;278(13):11465-11470. doi:10.1074/jbc.M210945200.

35. Mueller MM, Fusenig NE. Friends or foes - bipolar effects of the tumour stroma in cancer. Nat Rev Cancer. 2004 Nov;4(11):839-849. doi:10.1038/nrc1477.

36. Rupp C, Scherzer M, Rudisch A, et al. IGFBP7, a novel tumor stroma marker, with growth-promoting effects in colon cancer through a paracrine tumor-stroma interaction. Oncogene. 2015 Feb 12;34(7):815-825. doi:10.1038/onc.2014.18.

37. Bauer M, Su G, Casper C, He R, Rehrauer W, Friedl A. Heterogeneity of gene expression in stromal fibroblasts of human breast carcinomas and normal breast. Oncogene. 2010 Mar 25;29(12):1732-1740. doi:10.1038/onc.2009.463.

38. Tanaka S, Sugimachi K, Kameyama T, et al. Human WISP1v, a member of the CCN family, is associated with invasive cholangiocarcinoma. Hepatology. 2003 May;37(5):1122-1129. doi:10.1053/jhep.2003.50187.

39. Ono M, Inkson CA, Sonn R, et al. WISP1/CCN4: a potential target for inhibiting prostate cancer growth and spread to bone. PLoS One. 2013 Aug 14;8(8):e71709. doi:10.1371/journal.pone.0071709.

40. Feng M, Jia S. Dual effect of WISP-1 in diverse pathological processes. Chin J Cancer Res. 2016 Dec;28(6):553-560. doi:10.21147/j.issn.1000-9604.2016.06.01.

Отримано/Received 02.03.2021
Рецензовано/Revised 25.03.2021
Прийнято до друку/Accepted 05.04.2021

Information about authors

Dr. Gulhan Duman, MD, Assistant Professor, Division Endocrinology, Department of Internal Medicine, Sivas Cumhuriyet University, Faculty of Medicine, 58140, Sivas, Turkey; e-mail: gulcavlak@hotmail.com, contact phone: 0 (346) 2191010; fax: 0 (346) 2580000; https://orcid.org/0000-0002-4057-5701.

Baris Sariakcali, MD, Assistant Professor, Division of Endocrinology and Metabolism, Department of Internal Medicine, Cumhuriyet University Faculty of Medicine, 58140, Sivas, Turkey; e-mail: drbbarissariakcali@gmail.com; https://orcid.org/0000-0001-5133-1318.

Conflicts of interests. Authors declare the absence of any conflicts of interests and their own financial interest that might be construed to influence the results or interpretation of their manuscript.

Financial support. The authors declare that this study has received no financial support.

Ethical approval. The study protocol was approved by the Human Research Ethics Committee of Sivas Cumhuriyet University, Sivas, Turkey (Registry No: 2019-02/06). Our research was conducted ethically in accordance with the World Medical Association Declaration of Helsinki. Informed consent was obtained from all individual participants included in the study.

Authors contributions. GD conceived and designed the experiments. BS helped to gather data. GD wrote the first draft of the manuscript. BS contributed to the writing of the manuscript and agreed with manuscript results and conclusions. GD made critical revisions and approved final version. All authors reviewed and approved the final manuscript.

Gulhan Duman, Baris Sariakcali
Division of Endocrinology and Metabolism, Department of Internal Medicine, Cumhuriyet University Faculty of Medicine, Sivas, Turkey

Сироватковий рівень WNT-індукованого протеїну 1 як потенційний біомаркер тиреоїдних вузлів

Резюме. ***Актуальність.*** Вузли щитоподібної залози — поширені тиреоїдні захворювання у всьому світі, та їх частота значно зросла за останні десятиліття. Більшість тиреоїдних вузлів зазвичай випадково діагностуються як безсимптомні доброякісні утворення, виявлені методами візуалізації, проведеними з причин, не пов'язаних із захворюваннями щитоподібної залози. ***Метою*** даного дослідження було встановити значення рівня WNT-індукованого протеїну 1 (WISP1) у сироватці крові як допоміжного біомаркера для проведення диференціальної діагностики доброякісних та недоброякісних вузлів щитоподібної залози. ***Матеріали та методи.*** У дослідженні брали участь 89 пацієнтів, яким проведено тонкоголкову аспіраційну біопсію, та 43 особи контрольної групи. Серед обстежених жінки становили 72,7 % та 27,3 % — чоловіки. Вони були розподілені на дві групи відповідно до цитологічної оцінки Bethesda: доброякісні (Bethesda 2) та недоброякісні (Bethesda 3–6) утворення. Рівень WISP1 у сироватці крові вимірювали методом імуноферментного аналізу. ***Результати.*** У групі з доброякісними вузлами (Bethesda 2) були 58 (43,9 %) пацієнтів, та 31 (23,5 %) — у групі з недоброякісними (Bethesda 3–6) вузлами. Установлено, що розмір утворень був більшим у групі з недоброякісними вузлами, ніж у групі з доброякісними ($p = 0,006$). Рівень WISP1 у сироватці крові в групі хворих із доброякісними вузлами (Bethesda 2) був вірогідно вищим, ніж у групі з недоброякісними утвореннями (Bethesda 3–6) та осіб контрольної групи ($p < 0$). Різниця між хворими з доброякісними та недоброякісними вузлами відповідно до їх ехогенності була значущою ($p < 0,05$). У групі з доброякісними вузлами 76,9 % утворень мали змішану ехогенність, 76,7 % — були ізоехогенними, 68,4 % — ізогіпоехогенними та 35,7 % — гіпоехогенними. У хворих із доброякісними вузлами відзначалися найвища гіпоехогенність (64,3 %) і найменша змішана ехогенність (23,1 %). Не встановлено зв'язку між рівнями WISP1 та ехогенністю за допомогою критерію Kruskal-Wallis. ***Висновки.*** Згідно з результатами проведеного дослідження, вимірювання WISP1 у сироватці крові дозволяє отримати додаткову інформацію при диференціально-діагностичному аналізі пацієнтів із вузлами щитоподібної залози. Більш високий рівень сироваткового WISP1 дозволяє підтвердити цитологічний діагноз у хворих з доброякісними вузлами щитоподібної залози (Bethesda 2).

Ключові слова: вузли щитоподібної залози; ультразвукова діагностика; тонкоголкова аспіраційна біопсія; WISP1