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The Role of Serum Midkine and Secretory Leucocyte Protease Inhibitor in Diagnosis of Thyroid Cancer

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ABSTRACT

Introduction: The most frequent endocrine cancer is thyroid cancer. 95% of thyroid cancer cases are papillary thyroid carcinomas (PTC) and follicular thyroid carcinomas (FTC).

Aim: The study aimed to assess the value of serum midkine and secretory leucocyte protease inhibitor as noninvasive diagnostic markers for thyroid cancer. Serum levels of these markers concerning different thyroid cancer stages were also studied.

Patients and methods: This study included fifteen healthy controls with age and sex-matched thyroid cancer, and benign thyroid problem patients. Serum Midkine and secretory leucocyte protease inhibitor levels were measured by ELISA technique.

Results: Individuals with thyroid cancer had considerably greater midkine levels than patients with benign thyroid disorders and the control group. The SLPI was significantly higher in thyroid cancer patients compared to the control group. it was found that midkine had 88.4% accuracy with the area under curve 0.95 at cutoff point 895 pg/ml while SLPI had 61.2% accuracy with the area under curve 0.64 at cutoff point 1275.5 ng/ml.

Conclusion: Serum Midkine level and secretory leucocyte protease inhibitor are good markers for the detection of thyroid cancer with correlation with tumor stage.

Keywords: Thyroid nodule, Biomarker, Thyroid cancer, midkine, secretory leucocyte protease inhibitor.

INTRODUCTION

The most common endocrine cancer is thyroid cancer, and during the past few decades, its prevalence has grown [(1).] It is one of the 10 most common malignancies. 95% of thyroid cancer cases are papillary thyroid carcinomas (PTC) and follicular thyroid carcinomas (FTC) [(2).] When found in their early stages, PTC and FTC are frequently treatable, however, the chances of survival may drop from 100% in stages I and II to 50% in stages IV [(3).]

Therefore, the secret to effective therapy and a decrease in mortality is early identification. The results of pre-operative diagnostic procedures like ultrasonography, CT scans, and fine-needle aspirates (FNA) are frequently inconclusive. Up to 75% of patients who undergo diagnostic hemithyroidectomy surgery in these undetermined circumstances have a benign illness [(4).]

Consequently, biomarkers are required for diagnosis. Low molecular weight basic heparin-binding growth factor known as midkine (MK) [(5)]. MK affects cell proliferation, survival, migration, angiogenic, and antiapoptotic actions and is highly expressed throughout embryogenesis [(6).] A frequent characteristic of cancer is the overexpression of the MDK gene and the MK protein within the tumor.

SLPI is a newly developed serine protease inhibitor that promotes tumor growth and is overexpressed in several cancers [(7).] Protease inhibitors help initial tumor cells create networks resembling blood vessels and enhance perfusion. They are typically overexpressed in a variety of cancer tissues, such as those in the head and neck, breast, lung, and brain. **[(8)]**.

This study aims to evaluate the role of midkine and secretory leucocyte
protease inhibitor in differentiating malignant from the benign thyroid nodule. Studying serum levels of these markers with different thyroid cancer stages and histopathological types.

PATIENTS AND METHOD

This study was conducted on: a malignant group that included thirty patients diagnosed with thyroid cancer. (8 males and 22 females) their age ranged from 25 to 77 years. Another benign group included thirty patients with benign thyroid diseases (3 males and 27 females), and their ages ranged from 19 to 63 years. Also, twenty-five apparently healthy individuals were studied as a control group.

They included 6 males and 19 females. Their age ranged from 30 to 55 years. The study was conducted during the period from December 2020 to November 2021, the patients were chosen from the General Surgery Department of Assiut University Hospitals and South Egypt Cancer Institute. Patients and controls gave their formal consent. The Assiut University Faculty of Medicine's Ethical Committee gave its approval to the study.

Ethical Approval:

Each participant in the study provided written informed consent, which was obtained after the project was given the go-ahead by the Assiut University Ethics Board. The Declaration of Helsinki, the code of ethics of the World Medical Association, was followed when conducting this research.

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Classification of participants:

Thyroid cancer group: thirty patients were classified according to AJCC TNM staging into Grade I: which included 15 patients, Grade II: which included 8 patients, and Grade III: which included 7 patients . Based on histopathology; 23 patients had papillary carcinoma and 7 patients had follicular carcinoma. Ultrasound findings among thirty patients with benign thyroid diseases revealed that two patients had diffuse thyroid enlargement and 27 patients had multiple nodules.

There was one patient in this group who had a solitary thyroid nodule. Fine needle cytology of those patients revealed colloid nodular goiter, multinodular goiter, simple nodular goiter, and Hashimoto's thyroiditis in 13, 5, 10, and 2 patients, respectively. The control group included 25 apparently healthy persons, sex- and age-matched with both patients' groups.

All patients were subjected to fine needle aspiration biopsy and thyroid ultrasound. All subjects underwent comprehensive medical history, clinical examinations, and lab tests. Sample collection: Under strict aseptic conditions, ten milliliters of venous blood were taken and two milliliters of them were collected into an EDTA-coated tube for a complete blood count. The other eight ml were collected into two tubes containing clot activator and were centrifuged at a speed of 3000 rpm for 20 min for separation of serum. The serum was divided into aliquots and used for routine investigations and assay of Midkine and secretory leucocyte protease inhibitor levels.

Laboratory investigations: Serum urea nitrogen, serum creatinine, and liver functions were done on ADVIA1800, Siemens (Germany). Complete blood count: was done ADVIA 2120i Siemens (Germany).

Free T3, Free T4, and TSH were done on ADVIA Centaur XPT (Siemens, Germany).

Serum Midkine level: was measured by ELISA technique using human MDK ELISA Kit catalog no.: SG-10623, purchased from Sino GeneClon Biotech Co., (China). Serum secretory leucocyte protease inhibitor level: was measured by ELISA technique using SLPI ELISA Kit catalog no.: SG-11398, purchased from Sino GeneClon Biotech Co., (China)

RESULTS

Results of routine laboratory investigations are shown in **Table 1.**

Table 1: Laboratory data among studied groups

	Thyroid cancer (n= 30)	**Benign thyroid diseases (n= 30)**	**Control group (n= 25)**	***P* 1**	***P* 2**	***P* 3**
Leucocytes (10^9/l)	6.93± 1.27	7.28 ± 1.87	7.55 ± 1.73	0.49	0.25	0.61
Hemoglobin (g/dl)	11.8 ± 1.12	11.85 ± 1.12	11.68 ± 0.86	0.96	0.66	0.63
Platelets (10^9/l)	286 ± 8.29	313.6 ± 7.36	283.6 ± 6.76	0.17	0.88	0.15
Urea (mmol/l)	4.59 ± 1.06	4.88 ± 1.04	4.60 ± 0.99	0.28	0.98	0.31
Creatinine (µmol/l)	69 ± 6.87	65.14± 5.40	67.88± 5.85	0.25	0.65	0.53
Protein (g/l)	6.46± 0.71	6.72 ± 0.78	67.88± 15.85	0.43	0.92	0.40
Bilirubin (umol/l)	0.38± 0.09	0.42 ± 0.17	0.47 ± 0.17	0.47	0.07	0.25
AST (U/L)	22.8± 3.41	22.85± 3.75	22.72± 3.42	0.89	0.99	0.99
ALT (U/L)	23.1± 3.89	23.21± 5.86	23.16± 3.80	0.88	0.82	0.96
Albumin (g/l)	3.91± 0.76	4.14 ± 0.55	4.09 ± 0.65	0.87	0.09	0.22
ALP (U/L)	112± 12.87	118.7± 10.65	115.8± 12.87	0.98	0.08	0.11
TSH (mmlu/mL)	1.84± 0.19	1.61 ± 0.31	2.81 ± 0.04	0.46	**< 0.001**	**< 0.001**
Triiodothyronine (pg/ml)	3.14 ± 0.91	3.30 ± 0.76	3.15 ± 0.61	0.44	0.98	0.47
Thyroxin (ng/dl)	1.24 ± 0.36	1.31 ± 0.39	1.14 ± 0.20	0.42	0.30	0.07

Data expressed as mean (SD), range. P-value was significant if < 0.05.

AST: aspartate transaminase; ALT: alanine transaminase; TSH: thyroid stimulating hormone

P1 compares thyroid cancer and benign thyroid disease groups.

P2 compares thyroid cancer and control groups. P3 compares control and benign thyroid disease groups.

Patients with thyroid cancers had a significantly higher midkine in comparison to those with benign thyroid diseases and the control group (P <0.001 for both). Patients with benign thyroid disease had a significantly higher midkine in comparison to the control group. (P <0.001).

(**Table 2**), SLPI was significantly higher among patients with thyroid cancers in comparison to the control group (P = 0.01). There were no significant differences as regard SLPI between those with thyroid cancers and those with benign thyroid disease and between those with benign thyroid disease and the control group (P =0.12 and P =0.33, respectively) **(Table 2).**

Table 2: Midkine and secretory leucocyte protease inhibitor among the studied groups

	Thyroid cancer (n= 30)	Benign thyroid diseases (n= 30)	Control group (n= 25)	*P* 1	*P* 2	*P* 3
Midkine (pg/ml)	1033.7 ± 72.1	806.7 ± 56.7	549.9± 116.9	< **0.001**	< **0.001**	< **0.001**
SLPI (ng/ml)	1625.2 ± 96.1	1395.5 ± 85.6	1245.1 ± 45.9	0.12	**0.01**	0.33

Data expressed as mean (SD), range. *P*-value was significant if < 0.05. SLPI: secretory leucocyte protease inhibitor
*P*1 compares thyroid cancer and benign thyroid disease groups, *P*2 compares thyroid cancer and control groups; *P*3 compares control and benign thyroid disease groups.

Based on grades of thyroid cancers, patients with thyroid cancers grade II had a significantly higher midkine in comparison to those with grade I, and those with grade III had a significantly higher midkine in comparison to those with grade I (P <0.001 for both).

No significant difference was found between those with grade II and those with grade III (P=0.25). SLPI was significantly higher among patients with thyroid cancers grade III in comparison to those with grade I (P=0.01). No significant differences as regards SLPI between those with grade I and those with grade II (P=0.22) and between those with grade II and those with grade III (P=0.09) **(Table 3).**

Table 3: Midkine and secretory leucocyte protease inhibitor based on grades of thyroid cancers

	Grade I (n= 15)	Grade II (n= 8)	Grade III (n= 7)	*P* 1	*P* 2	*P* 3
Midkine (pg/ml)	893.6 ± 60.8	1146.1 ± 136.0	1205.2± 113.2	< **0.001**	< **0.001**	0.25
SLPI (ng/ml)	1327.5 ± 45.2	1665.6± 44.0	2216.8± 73.2	0.22	< **0.001**	0.09

Data expressed as mean (SD), range. P-value was significant if < 0.05. SLPI: secretory leucocyte protease inhibitor
P1 compares grade I and grade II groups,
P2 compares grade I and grade III groups
P3 compares grade II and grade III groups

Roc curve analysis was done to evaluate the diagnostic accuracy of midkine and SLPI in the detection of thyroid cancer **(Table 4)** and to differentiate between benign cases and stage I thyroid cancer to detect early thyroid cancer. **(Table 5).**

Table 4: Diagnostic accuracy of midkine and SLPI in the detection of thyroid cancers

	Midkine	SLPI
Sensitivity	83.3%	73.3%
Specificity	98.18%	54.5%
Positive predictive value	95.5%	46.8%
Negative predictive value	85.7%	78.9%
Accuracy	88.4%	61.2%
Cutoff point	895	1275.5
Area under curve	0.95	0.64
P-value	< 0.001	0.01

P-value was significant if < 0.05. SLPI: secretory leucocyte protease inhibitor

Table 5: Accuracy of midkine and SLPI in the detection of early thyroid cancers (early stage vs. benign diseases)

	Midkine	SLPI
Sensitivity	72.2%	44.4%
Specificity	93.6%	90.3%
Positive predictive value	86.7%	72.7%
Negative predictive value	85.3%	73.7%
Accuracy	88.7%	73.1%
Cutoff point	895	1332.3
Area under curve	0.86	0.66
P-value	< 0.001	< 0.001

P-value was significant if < 0.05. SLPI: secretory leucocyte protease inhibitor

Regarding SLPI at cutoff point 1275.5 ng/ml, had 61.2% accuracy, sensitivity 73.3%, specificity 54.5% with area under curve was 0.64, positive predictive value 46.8%, negative predictive value 78.9% and P value <0.01.

To detect early-stage of thyroid cancers (grade I), ROC curve analysis was done to differentiate between benign cases and stage I thyroid cancer. It was found that midkine at cutoff point 895 pg/ml, had 88.7% accuracy, sensitivity of 72.2%, specificity of 93.6% with an area under the curve was 0.86, positive predictive value of 86.7%, negative predictive value of 85.3% and P-value <0.001.

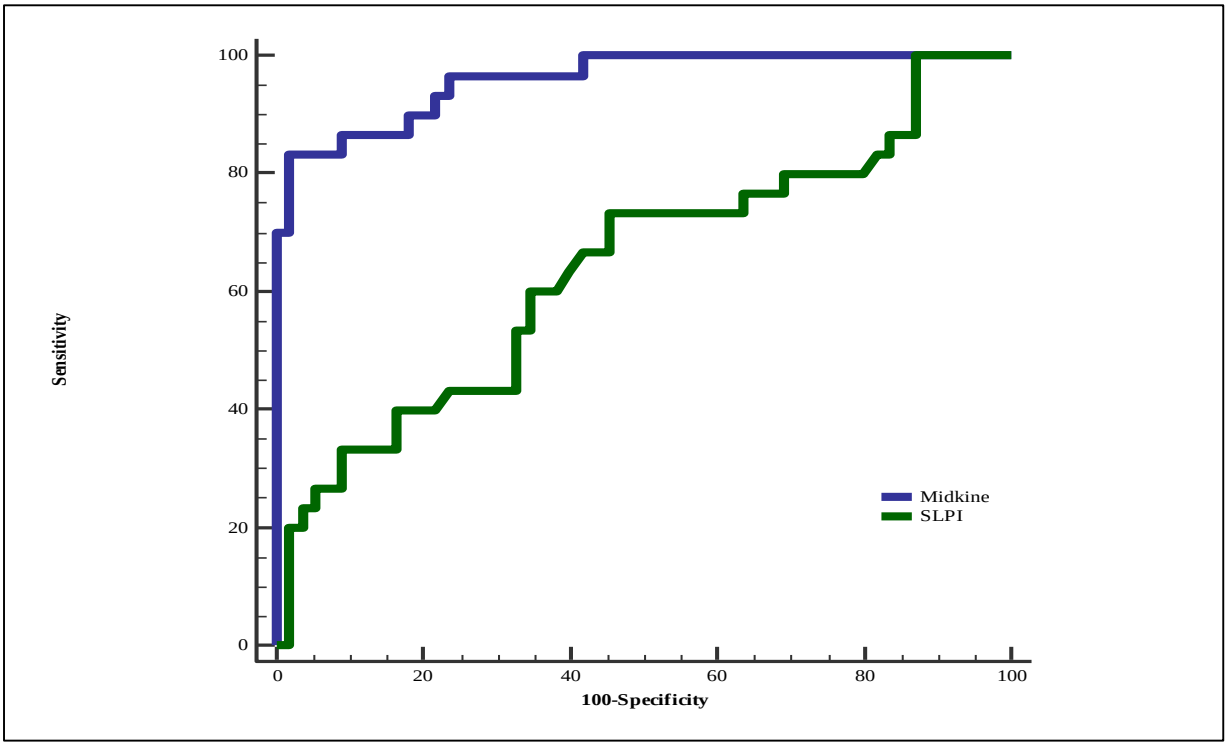

Figure 1: ROC curve analysis of midkine and SLPI in the diagnosis of thyroid cancers.

SLPI at cutoff point 1332.3 ng/ml, had 73.1% accuracy, sensitivity 44.4%, specificity 90.3% with area under curve was 0.66, positive predictive value 72.7%, negative predictive value 73.7% and P-value <0.001. **(Figure 2).**

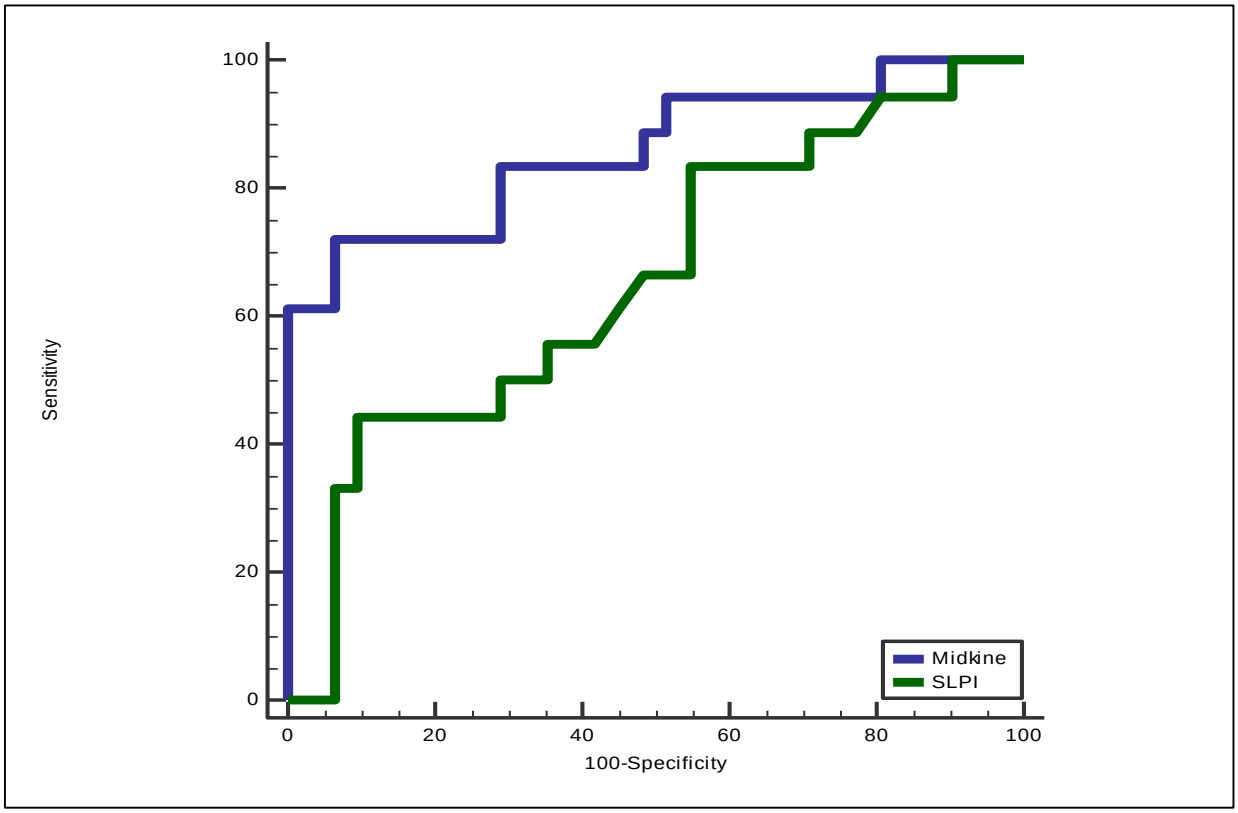

Fig. (2): ROC curve analysis of midkine and SLPI in the detection of early thyroid cancers

DISCUSSION

Genetics, family history, and prior radiation exposure are all risk factors for thyroid cancer. Two very efficient therapies include surgery and radio-iodine therapy. , but because thyroid cancer can be difficult to diagnose, surgery is frequently done on benign nodules. About 10 times more people have benign thyroid growths such as goiter, follicular adenomas, and cysts than thyroid malignancies [(9).]

The results of pre-operative diagnostic procedures like ultrasound, CT scans, and fine needle aspirations (FNA) are frequently inconclusive [(10).] Biomarkers are therefore required for diagnosis. A pleiotropic growth factor that is highly expressed during embryogenesis is called midkine (MK). It controls angiogenic, anti-apoptotic, and cell migration, growth, and survival [(11).] Because MK is a soluble cytokine that is readily detectable in blood circulation, it has the benefit of being a relatively practical and non-invasive biomarker [(12).] Midkine was released into the blood from cancerous tissue. SMK was discovered in 87% of different tumors. High blood and tissue MK levels have been postulated as predictive indicators of tumor activity and as indicators of malignancy in a variety of malignancies **[(13)]**.

In the present study, Midkine levels were noticeably greater in thyroid cancer patients compared to the control group and those with benign thyroid conditions. Additionally, compared to the control group, patients with benign thyroid illness had considerably greater midkine levels. According to the most recent research **Gebur and Ali, [(14)]** reported high plasma midkine levels in patients with malignant thyroid nodules.

The secreted serine protease inhibitor known as secretory leukocyte protease inhibitor (SLPI) is implicated in the development of tumors and is overexpressed in several malignancies [(15).] These inhibitors enable primary tumor cells to form vascular-like networks and improve perfusion. They are often overexpressed in a range of cancer tissues, including lung, brain, head/neck, and breast malignancies **[(16)]**.

According to the results of the current investigation, individuals with thyroid cancer had considerably greater SLPI levels than those in the control group. In contrast; there were no significant differences as regards SLPI between those with thyroid cancers and those with benign thyroid disease and between those with benign thyroid disease and the control group.

This was supported by **Stępień *et al.* [(8)]** They reported that patients with PTC had considerably greater SLPI concentrations than MNG and controls. Additionally, they discovered that there was no discernible difference in SLPI levels between MNG and healthy controls. Based on grades of thyroid cancers, a comparison of Midkine and secretory leucocyte protease inhibitor between the studied groups revealed that patients with thyroid cancers grade II had significantly higher midkine in comparison to those grade-I and those with grade III had significantly higher midkine in comparison to those grade-I. But no significant difference was found between those with grade II and those with grade III. SLPI was significantly higher among patients with thyroid cancers grade- III in comparison to those with grad-I. In contrast; there were no significant differences as regards SLPI between those with grade-I and those with grade II and between those with grade II and those with grade III.

This was supported by **Zhou *et al.* [(17)] and Ibrahim and Hamam [(18)]** who found that MK expression significantly differed according to TNM staging. Furthermore, a high amount of midkine expression has been linked to a bad prognosis and an advanced tumor stage.

Regarding secretory leucocyte protease inhibitor, **Stępień *et al.* [(8)]** is the first and only study to show that patients with all histologic subtypes of PTC had higher serum levels of SLPI. The serum levels of SLPI were the same as those seen in the group of MNG patients with benign tumors. These data imply that elevated SLPI concentrations are linked to thyroid epithelial cell oncogenesis at an early stage **[(19)]**.

Using ROC curve analysis, we found that, for diagnosis of thyroid cancers, serum midkine at cutoff point 895 pg/ml, had 88.4% accuracy, sensitivity of 83.3%, specificity of 98.18% with area under curve 0.95, positive predictive value of 95.5%, negative predictive value 85.7% and P value <0.001.

As well, **Ibrahim and Hamam, [(18)]** showed that the measurement of serum midkine in solitary thyroid nodules resulted in an Area under the curve (AUC) in the ROC curve of (AUC = 0.875, P 0.001). To identify suspicious or malignant nodules, a serum midkine threshold value of 0.68 ng/mL was determined to have a 76% sensitivity and 86% specificity. Another study by **Meng *et al.* [(20)]** discovered that midkine has a workable diagnostic capacity to distinguish benign thyroid nodules from DTC before surgery (cutoff value: 323.12 pg/ml, diagnostic accuracy: 75.31%).

CONCLUSION

This study has shown that both serum midkine and Patients with thyroid cancer had higher levels of secretory leucocyte protease inhibitor than healthy people. The thyroid nodules on the body can be classified as benign or malignant by serum midkine. with correlation with tumor grade. Serum midkine was better than secretory leucocyte protease inhibitor in the diagnosis and early detection of thyroid cancer with higher sensitivity and specificity and diagnostic accuracy.

DECLARATIONS

Consent for Publication: I attest that all authors have agreed to submit the work.

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