ORIGINAL ARTICLE

Estimation of Serum Thyroid Stimulating Hormone and Anti-Thyroid Antibodies as Biomarkers of Malignancy in Thyroid Nodule Patients

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ABSTRACT

Aim: To find out association of Serum Thyroid Stimulating Hormone (TSH) and Anti-Thyroid Antibodies (ATA) i.e., anti Tg-Ab & anti TPO-Ab with thyroid carcinoma in thyroid nodule patients presented in Mayo Hospital Lahore.
Study design: It was a comparative cross-sectional study.
Methods: It wasconducted in Biochemistry Department and Department of Biomedical Sciences KEMU, Lahore from February 2018 to May 2019. Seventy patients were included in the study based on selection criteria and data was recorded on a preformed questionnaire. The study participants were classified into benign (n=36) and malignant categories (n=34) based on histopathological & radiological investigations. Serum levels of TSH and anti- thyroid antibodies (TPO-Ab&Tg-Ab) were measured employing ELISA (Enzyme Linked Immunosorbent Assay) technique.
Results: Thyroid nodules were found to be more prevalent in females (n=64, 91.4%). The mean age of the study subjects was 38.46±12.92 years and there was a significant association of older age with thyroid carcinoma (p=0.001). Moreover, Serum TSH, Anti-Tg-Ab and TPO-Ab were higher in malignant than in benign thyroid nodules and were significantly associated with thyroid cancer (p=<0.05). Furthermore, a binary logistic regression analysis concluded that higher serum TSH levels may increase the risk of malignancy in thyroid nodule patients (OR=5.124).
Conclusion: Older age and higher levels of serum TSH, Tg-Ab and TPO-Ab showed a significant association with thyroid carcinoma in thyroid nodule patients.
Keywords: Thyroid carcinoma, Thyroid stimulating hormone, Anti- thyroglobulin Antibody, Anti-TPO antibodies, Thyroid nodule

INTRODUCTION

The most common endocrine malignancy is thyroid carcinoma with an annual incidence of 0.5 to 10 per 100,000 subjects in world population[1]. An increased incidence of thyroid cancer has been reported recently and it ranks highest after breast cancer in women[2]. Earlier detection of the subclinical disease with ultrasound and ultrasound guided fine needle aspiration biopsy (FNAB) resulting in the detection of asymptomatic disease may be the reason for this increased incidence of thyroid carcinoma[3,4]. The factors responsible for the cancer are still not well known. However, certain risk factors have been identified over the years that includes age, female gender, exposure to radiation and family history of thyroid cancer[5-8]. In Pakistan Papillary Thyroid Carcinoma is the most common thyroid malignancy. The symptoms of thyroid cancer usually include neck swelling that has recently increased in size, voice change and difficult swallowing and breathing. These are mostly multinodular goiters. About 3% of multinodular goiters and 4.4% of solitary nodules of the thyroid are malignant[9].

Association of increased serum TSH with thyroid carcinoma is possibly because of its role in affecting thyroid cell differentiation and proliferation or in stimulating angiogenesis[10].Moreover, higher TSH levels have also been found to be associated with advanced cancer stage so it has been hypothesized that TSH may play a vital role in thyroid cancer progression[11].

Recently, thyroid autoimmunity has also been reported as a risk factor for thyroid malignancy. The hallmark of thyroid autoimmunity is the presence of anti-thyroid antibodies (ATA) i.e, anti-thyroglobulin antibodies (Tg-Ab) and anti-thyroperoxidase antibodies (TPO-Ab)[12]. A higher prevalence of thyroid carcinoma is found in patients with positive serum Tg-Ab and/or TPO-Abthan in patients with negative anti thyroid antibodies[13]. Proposed mechanism for malignancy with autoimmunity is that chronic antigenic stimulation may cause neoplastic hyperplasia of thyroid follicles and thus malignant transformation[1].

The prognosis of thyroid carcinoma is good if detected early and resected. Surgeons face a significant challenge in identifying thyroid nodule patients who require aggressive surgical treatment for carcinoma. Fine-needle aspiration cytology (FNAC) is the gold standard for evaluating patients with thyroid nodules[14]. It has a high specificity and sensitivity for evaluating whether a nodule is benign or malignant and remains the mainstay for the diagnosis of thyroid carcinoma[15]. FNAC specimen are read by an experienced cytopathologist and be reported according to The Bethesda System for Reporting Thyroid Cytology (TBSRTC) with six diagnostic categories[14]. Diagnostic Surgery is indicated for FNAC findings of indeterminate cytology (Bethesda category III) or suspicious for differentiated thyroid carcinoma (Bethesda category IV/V)[16]. Clinicians have been looking for diagnostic biomarkers to predict the risk of carcinoma in patients with indeterminate cytology or suspicion of malignancy on FNAC to avoid unnecessary diagnostic surgeries. Serum TSH and ATA may serve as supplementary biomarkers and discriminate benign from malignant nodules in combination with FNAC results.

It may also help surgeons to make timely decisions and identify patients with thyroid nodules at greater risk of developing thyroid carcinoma, which may improve the prognosis of the disease.

METHODS

It was a comparative cross-sectional study conducted at Departments of Biochemistry and Biomedical Sciences of KEMU in collaboration with the Pathology and Surgery Departments of Mayo Hospital Lahore from February 2018 to May 2019. Sample size of 70 patients was estimated using 5% level of significance, 95% power of test with expected mean value, thyroid cancer benign group as 2.48 ± 1.65 and malignant group as 4.76±2.43[9].

$$n= \frac{\sigma^2(Z_{1-\alpha/2} + Z_{1-\beta})^2}{(\mu_o - \mu_a)^2}$$

$\sigma^2 =$	Variance	=	4.08
$Z_{1-\alpha/2} =$	Confidence level 95% =		1.96
$Z_{1-\beta} =$	Power of test	=	95%

$\mu_o=$ Population mean 1 =   2.48
$\mu_a=$ Population mean 2 =   4.76

A total of two hundred and thirty-two (n=232) patients with goitre were presented to the surgery and pathology departments during our study period. 70 of the 232 patients with predominant solid nodules, ranging in age from 20 to 70 years, were chosen for further analysis and diagnosis via thyroid function tests, a thyroid pertechnetate scan, a diagnostic thyroid ultrasound, and FNAC. Patients with a prior history of thyroid surgery, hyperthyroidism, or head and neck irradiation were excluded. Ethical approval of our research project was obtained from the IRB of KEMU. After thoroughly explaining the research project, the study subjects provided written and informed consent. The procedure for collecting blood was explained to the selected patients. Then, using standard phlebotomy techniques, 5 ml of blood was drawn. The operative procedure was carried out under general anesthesia. The pathology department received the resected thyroid specimen to determine the final diagnosis of the thyroid nodule. Based on their report, study subjects were placed into benign and malignant groups. There were 36 benign and 34 malignant thyroid nodule patients. Serum levels of TSH and anti-thyroid antibodies (TPO-Ab and Tg-Ab) were determined by using the ELISA (enzyme-linked immunosorbent assay) technique.

Data was analyzed in SPSS-20 (Statistical Package for Social Sciences). Quantitative variables like age, TSH, Tg-Ab, and TPO-Ab were presented as mean ± SD. The comparison of quantitative variables amongst patients in the benign and malignant groups was done by employing the Mann-Whiteny U test if the data was not in normal distribution. Spearman's correlation analysis was carried out to find the association between serum levels of TSH and anti-thyroid antibodies in both groups, i.e., benign, and malignant. Both a binary logistic regression analysis and a Spearman correlation analysis were carried out to ascertain the relationship between thyroid cancer and serum TSH, Tg-Ab, and TPO-Ab. It was considered significant at a 0.05 p-value.

Amongst the study subjects, the majority were females 64(91.4%). The mean (±SD) age of included subjects was 38.46 (±12.92) years, and the majority had a normal BMI 53(75.7%) (Table 1).

Table 1: Demographic characteristics of Thyroid Nodule Patients

Demographic characteristics	n	%age
Gender		
Male	6	8.6
Female	64	91.4
Age (years) Mean±SD 38.46±12.92		
21-30	25	35.7
31-40	18	25.7
41-50	18	25.7
>50	9	12.9
BMI		
Underweight	3	4.3
Normal	53	75.7
Overweight	13	18.6
Obesity	1	1.4

The difference in age and serum TSH, Tg-Ab, and TPO-Ab levels between patients with benign and malignant thyroid nodules was statistically significant (p<0.05), meaning that thyroid carcinoma patients had higher serum levels of these markers and were older than patients with benign thyroid nodule. (Table 2)

In order to quantify the strength of the monotonic relationship statistically, correlation analysis (Spearman's) showed a strong positive correlation (r_s =0.695) between TSH and thyroid cancer, a moderate positive correlation (r_s =0.431 & r_s =0.529) between Tg-antibodies and thyroid carcinoma, and a weak positive correlation (r_s=0.362, r_s= 0.228, and r_s = 0.224) between TPO-antibodies and thyroid carcinoma (Table 3).

A simple binary logistic regression analysis was carried out to predict the relationship between independent variables i.e; serum biomarkers in our study- TSH, Tg-Ab& TPO-Ab and Odds ratio in favor of thyroid carcinoma was calculated.

RESULTS

Table 2: Association of Thyroid Carcinoma with Age, Serum TSH, Tg-Ab& TPO-Ab

Variables	Median (IQR)		Mann-Whitney U Test (p-value)
	Benign	**Malignant**	
Age (years)	30 (25.25-40.00)	45(35.00-50.00)	0.001*
TSH (mIU/L)	3.02 (2.52-3.375)	4.79(3.86-6.02)	0.000*
Tg-Antibodies	58.40 (52.75-11.75)	164.00(131.75-318.25)	0.000*
TPO-Antibodies	33.50 (29.25-66.08)	65.30(35.30-481.53)	0.003*

*A p-value <0.05 was considered significant

Table 3: Correlation of biomarkers with Thyroid Carcinoma

Serum biomarkers	Thyroid Carcinoma	TSH	Tg Antibodies
TSH	0.695***	-	-
Tg-Antibodies	0.431**	0.529**	-
TPO-Antibodies	0.362*	0.228*	0.224*

***Strong positive correlation,   **Moderate positive correlation,   *Weak positive correlation

Table 4: Simple Binary Logistic Regression analysis in prediction of Thyroid Carcinoma

Predictor Variable	OR for Thyroid Carcinoma	95% CI for OR	p-value
TSH	5.124	2.136-12.293	.000*
Tg-Ab	1.002	.997-1.006	.403
TPO-Ab	1.000	1.000-1.001	.565

A p-value < 0.05 was considered significant

DISCUSSION

During clinical practice, thyroid nodules are frequently noticed. About 8-15% of them have a chance of being cancerous[17], while the majority are benign. If the proper workup and appropriate management in the right direction are carried out in due time, thyroid cancer has a very good prognosis. Patients with FNA suspicious for malignancy cannot be treated in the same way as those with benign or malignant cytology that is unambiguously benign or malignant. Therefore, to avoid these patients from undergoing neck diagnostic surgeries, we also need to use some other biomarkers in addition to FNAC.

Our study confirmed findings from numerous international studies that thyroid nodules are clearly more common in females[11,17,18].

An Italian study finding that thyroid cancer risk increases with age[19] came to the same conclusion that the malignancy was common among older age groups and that there was a significant age difference between benign and malignant patients.

However, according to another study, there was a greater risk of developing carcinoma in younger patients than in older ones.[18]This might be because study participants ranged in age from different age groups.

Recently, TSH has also been reported to play a significant role in thyroid cancer development and progression. In our study,

we compared serum TSH among benign and malignant groups. We found that serum levels of TSH were higher in thyroid carcinoma patients than in benign thyroid nodule patients. Our results have been consistent with the study conducted in USA which revealed that thyroid cancer was more prevalent in patients who had higher serum TSH levels[20]. A research project conducted in 2017 highlighted the importance of serum TSH as the biomarker of malignancy[21].

Anti-thyroid antibodies (Tg-Ab& TPO-Ab) have also being considered to play a role in thyroid cancer development and are an emerging biomarker for thyroid carcinoma. In our study the relationship between anti-thyroglobulin antibodies and thyroid carcinoma had also been examined and we found that Tg-Ab was significantly associated with thyroid cancer. Tg-Ab levels were higher in patients with thyroid cancer than in benign thyroid nodules. Our results have been supported by Chinese researchers who found that serum Tg-Ab was an independent risk factor for thyroid carcinoma[22]. Moreover, recently it was observed by other investigators that increased serum Tg-Ab levels were associated with thyroid cancer[23].

In addition to this TPO-Ab levels were also compared among benign and malignant nodules, and it was found that higher levels of TPO-Ab were present in patients with malignant nodules than in benign nodules. Our results have been supported by a study carried out in 2019 which determined that higher levels of TPO-Ab were linked with thyroid carcinoma[24].

The results of a spearman correlation analysis were consistent with the data from studies that mentioned serum TSH, Tg-Ab levels were positively correlated with thyroid cancer[21,25], but TPO-Ab levels were not correlated to thyroid cancer in a recent study[26]. Contrary to this finding, there was a weak positive correlation between TPO-Ab and thyroid cancer in our study.

A binary logistic regression analysis was carried out to predict the relationship between independent variables, serum TSH, serum Tg-Ab and Odds ratio in favor of thyroid carcinoma was calculated. A p-value<0.05 showed that there was a statistically significant effect of serum TSH on malignancy (B=1.634). The value of odds ratio (OR=5.124) reflects that one-unit increase in serum TSH may cause 5 times higher risk of thyroid cancer. So, serum TSH turned out to be a significant predictor of thyroid cancer in thyroid nodule patients as described in a study conducted in USA[20]. While Tg-Ab (OR=1.002) and TPO-Ab (OR=1.00) were not considered the significant predictors of thyroid cancer. However, a recent Chinese study determined that thyroid carcinoma was significantly associated with Tg-Ab(OR = 2.1) and not with TPO-Ab (OR = 1.20). This meta- analysis conducted in 2019 concluded results from different studies carried out on thyroid carcinoma and antithyroid antibodies, established Tg-Ab an independent risk factor for thyroid carcinoma while TPO-Ab needs further studies to determine its role as a biomarker for thyroid carcinoma[26]. This difference of results might be due to different detection techniques, cut off values of kits used and small sample size.

CONCLUSION

In this study, we discovered that thyroid cancer was significantly associated with advancing age and higher serum levels of TSH, Tg-Ab, and TPO-Ab. TSH and Tg-Ab have been shown in numerous international studies, including our study, to be predictive biomarkers of malignancy, but there is little information available on their utility in Pakistan. However, our study may contribute to the body of knowledge in the country, help avoid unnecessary diagnostic procedures, and help identify thyroid nodule patients who are more likely to develop thyroid cancer.

Recommendation: Results may not be generalize to a larger scale because the current study only included a small number of participants. Therefore, in the future, large sample size studies should be conducted to further develop and validate these findings.

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