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Original Article

Relationship between thyroid nodule, prolactin, and thyroid peroxidase antibody levels: A case-control study

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Abstract

Background: Thyroid nodules are a significant clinical concern due to their potential for malignancy. Research suggests prolactin, a known mitogen, may contribute to cancer development, and elevated thyroid peroxidase antibody (TPO-Ab) levels are linked to increased thyroid nodularity in hyperprolactinemia. This study investigates the relationship between serum prolactin levels, thyroid nodularity, and TPO-Ab presence.

Methods: Ninety patients were divided into three groups of 30: a control group without nodules, and two case groups with nodules (one TPO-Ab positive, one TPO-Ab negative). Serum prolactin, TPO-Ab, thyroid stimulating hormone, T4, and thyroid ultrasound data were analyzed. Statistical significance was set at $p < 0.05$.

Results: The mean age of the participants was 46.07±12.40 years, with 16 (17.8%) males and 74 (82.2%) females. Serum TSH, prolactin, and TPO-Ab levels were significantly higher in the case group than in the control group (P=0.041, 0.050, and 0.000, respectively). The case group had more instances of hyperprolactinemia than the control group (35% vs. 13.3%) (OR = 3.5, CI 95% = 1.08-11.38, P=0.031). Furthermore, the TPO-Ab positive group demonstrated greater solidity than the TPO-Ab negative group (93.3% vs 73.3%, P=0.038).

Conclusion: Our study revealed a positive correlation between thyroid nodules and increased serum TSH, prolactin, and TPO-Ab levels. Furthermore, we discovered that TPO-Ab positive nodules exhibit greater solidity and elevated TSH levels.

Keywords: Thyroid nodule, Prolactin, Antithyroid peroxidase antibody (TPO-Ab), Thyroid-stimulating hormone (TSH).

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Thyroid nodules are a frequent clinical occurrence, and the primary concern associated with their diagnosis is to exclude the possibility of malignancy (1, 2). Despite the high prevalence of thyroid nodules, with over 50% of individuals having nodules larger than 1 cm, according to autopsy studies, the clinical detection rate is relatively low, with only 4%–7% of nodules being palpable (1). Ultrasound is the preferred modality for evaluating thyroid nodules, while nodules exhibiting suspicious features of malignancy necessitate cytological assessment (2). Fine-needle aspiration biopsy (FNAB), guided by ultrasound, is the preferred tissue sampling method (3). Nevertheless, approximately 25-30% of thyroid nodules are reported as indeterminate in cytology, necessitating further evaluations for surgical decision-making (4). Molecular genetic biomarkers are currently employed to improve the accuracy of thyroid cytology and potentially alter the clinical decision-making process (5, 6). However, the availability of molecular genetic biomarker evaluation still needs to be improved (7). Hence, alternative approaches are required to determine the risk of malignancy in thyroid nodules exhibiting indeterminate cytology.

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Prolactin, a 23 kDa polypeptide hormone comprising 198 amino acids, is synthesized in the lactotroph cells of the anterior pituitary gland. Hyperprolactinemia and cancer relationship has been a controversial topic for a long time. Much evidence is available about the role of prolactin in various types of cancer (reproductive and non-reproductive) through its local production or accumulation. Prolactin may have an anti-apoptotic effect, and experimental studies have shown that prolactin stimulates the proliferation of prostate and breast cells and, therefore, can be associated with prostate and breast malignancy (8, 9). Serum prolactin may be related to thyroid nodules and malignancy, so this study was designed to evaluate the relationship between a thyroid nodule and serum prolactin.

Methods

The present study is a case-control study, all new cases of this case-control study aimed to investigate the relationship between thyroid nodules and serum prolactin levels. The study participants were recruited from academic endocrinology clinics in Sari. The research participants were categorized into two distinct groups: the case group and the control group. Within the case group, two subgroups were delineated patients with thyroid nodules and normal TSH levels (with or without drug treatment) and positive Anti-TPO, and individuals with thyroid nodules, normal thyroid test results, and negative Anti-TPO. The control group had normal thyroid tests without thyroid nodules and negative Anti-TPO. Inclusion criteria included age between 20 and 70 years and normal TSH (with or without treatment), while exclusion criteria encompassed pregnancy and breastfeeding, hepatic or renal failure (Cr>2 mg/dl), polycystic ovarian syndrome, hyperandrogenism, and the use of medications affecting prolactin levels. Ethical approval for the study protocol was obtained from the Mazandaran University of Medical Sciences Ethics Committee (IR.MAZUMS.IMAMHOSPITAL.REC.1397.094), and informed consent was obtained from all 90 eligible patients. The case group was patients with thyroid nodules (30 with positive and 30 with negative anti-TPO). The control group consisted of 30 patients without thyroid nodules, regular thyroid function tests, and negative anti-TPO. Serum prolactin levels were measured in all three groups. To prove the presence of thyroid nodules, a thyroid ultrasound with a 7.5 MHz linear probe and an Alpinion Ecabe 15 device made in Korea was used. Ultrasound was performed by one of the colleagues of the project who did not know about the groups. Thyroid nodules with a size of more than one centimeter were examined. Sonographic characteristics of the nodule, solid or cystic, echogenicity, border of the nodule, internal calcification of nodule, single or multiple, extension outside the thyroid, taller than wide and report of multiple lymphadenopathy in the six regions of the neck were of interest.

Prolactin measurement method: Prolactin levels fluctuate throughout the day, with a peak during sleep and after waking up. Various factors can temporarily increase prolactin levels, including physical and emotional stress, a high protein diet, breast stimulation, and a recent breast examination. To accurately measure prolactin levels, patients were tested in the morning, 2 hours after waking up, and after refraining from exercise. TSH, TPO Ab, and prolactin were measured by the ELISA method using an Ernest device made in America with a Belgian kit.

Statistical analysis: To compare prolactin levels between groups, independent t-tests, and ANOVA were utilized. The chi-square test was used for the comparison of qualitative data. Pearson's or Spearman's correlations were used to evaluate the correlation between prolactin and thyroid function tests based on the normality of data distribution.

Results

Ninety euthyroid patients in three groups: 30 with a thyroid nodule and positive anti-TPO, 30 with a thyroid nodule and negative anti-TPO (as case group), and 30 people without thyroid nodule and negative anti-TPO (as the control group) were enrolled in the present study. Fifty-eight women and thirty-two men were studied. The gender distribution was not statistically different between groups (P= 0.372) (table 1). Mean age, TSH, and serum prolactin levels were compared between two groups with and without nodules. Other variables based on the Kolmogorov-Smirnov test did not have a normal distribution except age. Therefore, an independent t-test was used for data with normal distribution and a Mann-Whitney U test for non-normal distribution to compare the average of the above variables. TSH, serum prolactin, and TPO-Ab levels were significantly higher in the case group than in the control group (P =0.041, 0.050, and 0.000, respectively) (table 2). The ANOVA test compared the age, TSH, and serum prolactin levels between the three groups. Only the TSH level showed a significant difference between the groups. Since the homogeneity of variances test for TSH was insignificant, Bonferroni's post hoc test was used to compare the two groups. TSH level was significantly higher in both case subgroups (TPO-Ab positive and negative) compared to the control group (P=0.007 and P=0.032, respectively). Although the amount of serum prolactin was

higher in the case than in the control group (19.44±12.38 vs. 14.64± 7.96 ng/ml), this difference was not statistically significant (P=0.05). Hyperprolactinemic (prolactin >20 ng/ml) cases number significantly higher among the case group (35%) compared to the control group (13.3%) (OR = 3.5 CI 95% = 1.08-11.38, P=0.031). The comparison of hyperprolactinemic case numbers was not statistically different between the two case subgroups (TPO-Ab positive and negative subgroups, P=0.787). Forty-one (68.3%) patients in the case group and 23 (76.7%) patients in the control group used levothyroxine, which difference was not statistically significant (P=0.411). However, in the case of the group, a significant difference was observed between the two subgroups (TPO-Ab positive and negative), and more patients in the TPO-Ab positive group used levothyroxine (P=0.027). The comparison of ultrasound findings between two subgroups with TPO-Ab positive and TPO-Ab negative nodules is presented in Table 3, and the solidity was higher in the TPO-Ab positive than TPO-Ab negative group (93.3 % vs. 73.3% P=0.038).

Table 1. Gender distribution of patients in subjects with or without thyroid nodules

	Group				
Sex	**Control**	**Nodule + and TPO-Ab -**	**Nodule + and TPO-Ab +**	**Total**	**P-value**
Male	3 (10%)	6 (20%)	7 (23.3%)	16 (17.8%)	0.372
Female	27 (90%)	24 (80%)	23 (76.7%)	74 (82.2%)	

Table 2. Average age, TSH, T4 and serum prolactin levels between the two groups with or without nodules

	Group		
Parameter	**Negative nodule (mean ± standard deviation)**	**Positive nodule (mean±standard deviation)**	**P-value**
Age (year)	45.43± 11.24	46.40±13.2	0.730
TSH (mIU/L)	2.48±1.48	3.35±2.04	0.041
T4 (µg/L)	8.88±2.37	8.31±1.53	0.199
TPO-Ab	12.95±29.59	139.58±186.07	<0.0001
Prolactin (µg/L)	14.64±64	19.44±12.38	0.05

Table 3. Ultrasonographic comparison of nodules in patients with positive and negative TPO antibodies

		Nodule positive and TPO-Ab negative	**Nodule positive and TPO-Ab positive**	**Total**	**P-value**
Nodule type	Single	12 (40%)	14 (46.7%)	26 (43.4%)	0.602
	Multiple	18 (60%)	16 (53.3%)	34 (56.7%)	
Border irregularity	Yes	1 (3.3%)	2 (6.7%)	3 (5%)	0.990
	No	29 (96.7%)	28 (93.3%)	57 (95%)	
Calcification	Yes	13 (43.3%)	12 (40%)	25 (41.7%)	0.793
	No	17 (56.7%)	18 (60%)	35 (58.3%)	
Calcification type	Microcalcification	10 (76.9%)	5 (41.7%)	15 (60%)	0.111
	Macrocalcification	3 (23.1%)	7 (58.3%)	10 (40%)	
Hypoecho	Yes	8 (26.7%)	15 (50%)	23 (38.3%)	0.063
	No	22 (73.3%)	15 (50%)	37 (61.7%)	

		Nodule positive and TPO-Ab negative	Nodule positive and TPO-Ab positive	Total	P-value
Solid	Yes	22 (73.3%)	28 (93.3%)	50 (83.3%)	0.038
	No	28 (26.7%)	2 (6.7%)	10 (16.7%)	
Extrathyroidal solid extension	Yes	0 (0%)	0 (0%)	0 (0%)	1
	No	30 (100%)	30 (100%)	60 (100%)	
Taller than wide nodules	Yes	1 (3.3%)	0 (0%)	1 (1.7%)	0.990
	No	29 (96.7%)	30 (100%)	59 (98.3%)	

Discussion

In the present study, we examined three possible predictors of malignancy in thyroid nodules with normal TSH (with or without treatment), including serum prolactin, and TSH thyroid autoimmunity (TPO-Ab). The study compared serum TSH, prolactin, and autoimmune thyroid antibody levels among patients with thyroid nodules and normal TSH (with or without drug treatment) and positive Anti-TPO and the second group were individuals with thyroid nodules, normal thyroid tests, and anti-TPO negative as a case group and the control group who did not have thyroid nodules with normal TSH and had negative anti-TPO. The findings of the study revealed a significantly higher number of hyperprolactinemic cases (prolactin more than 20 ng/ml) in euthyroid patients with thyroid nodules compared to the control group who were euthyroid and had no nodule in thyroid ultrasound (35% vs. 13.3%) (OR = 3.5 CI 95% = 1.08-11.38, P=0.031). Prolactin can exert its effects on tissues through autocrine or endocrine mechanisms. The proliferative/antiapoptotic role of autocrine/paracrine prolactin has been confirmed by pharmacological approaches, and it has been shown that prolactin receptor (PRLP) antagonists partially inhibit the growth of breast and prostate cancer cell cultures. Although the relationship between hyperprolactinemia and cancer is contentious, recent studies have extensively investigated this association (10).

Limited research has been conducted on the correlation between prolactin, thyroid nodules, and thyroid cancer. Kedzie and colleagues reported that prolactin messenger RNA (mRNA) was present in wild mice's thyroid and parathyroid tissues, which showed that prolactin could regulate thyroid function. The authors also demonstrated the expression of prolactin receptor mRNA in thyroid follicles and calcitonin-producing C cells, suggesting a potential role of prolactin in the development of thyroid tumors (11). Lu et al. conducted a study investigating the relationship between hyperprolactinemia and calcitonin secretion in thyroid C cells in rats. The results indicated that hyperprolactinemia stimulates calcitonin secretion by activating the cAMP pathway through the direct effect of prolactin. Additionally, some researchers have suggested that prolactin can indirectly affect the expression of CD40 in thyrocytes. The opposing effects of interferon-gamma and interleukin 4 influence the intricate relationship between prolactin and thyroid function. Nevertheless, additional investigations are required to understand the underlying mechanisms (5) thoroughly.

In their study, Costa et al. utilized RT-PCR and immunohistochemistry techniques to demonstrate, for the first time, the expression of prolactin receptors in the human thyroid. This finding implies that the thyroid gland may be a target for prolactin in physiological or pathological hyperprolactinemia (12). The data of the present study, in line with the study of Abbas Ali Tam (10) et al. and Sayki et al. (13) and in contrast with the data of the study of T-Pilli et al. (16), could show the relationship between hyperprolactinemia and thyroid nodularity. In a survey conducted by Abbas Ali Tam et al. in 182 patients with prolactinoma compared to the control group, thyroid size, and nodularity were significantly higher in patients with prolactinoma (10) . In another study, Sayki et al. demonstrated that individuals with hyperprolactinemia are more likely to develop thyroid nodules, autoimmune disorders, and increased thyroid volume (13). In 2019, T-Pilli et al. conducted a retrospective case-control study in Italy to investigate the prevalence of autoimmune thyroid diseases in prolactinoma patients compared to a control group. The study aimed to identify any potential relationship between the presence of prolactinoma and autoimmune thyroid diseases. Notably, serum prolactin levels were identified as the sole independent predictor of

autoimmune thyroid disease, with thyroid size and multinodular goiter demonstrating no significant difference between the prolactinoma and control groups (14). Prolactin has an autocrine or paracrine effect on the target tissue. In the case of prolactinoma, lactotrophic cells of the pituitary gland secrete prolactin independently, which affects thyroid cells in a paracrine manner. On the other hand, in non-prolactinoma patients with hyperprolactinemia, other sources of prolactin, such as immune cells, may be involved, leading to an autocrine effect (10).

Patients may exhibit normal prolactin levels but altered prolactin receptor expression in some cases. Similar changes in the face of prolactin and its receptor have been observed in breast and other types of cancer. The biological effects of prolactin are mediated through its receptor, a member of the hematopoietic cytokine receptor family. Upon binding to its receptor, prolactin activates several signaling pathways, including JAK/STAT, MAPK, and PI3K, resulting in cell differentiation, proliferation, survival, and secretion. Animal and in vitro studies have demonstrated that prolactin promotes tumorigenesis by stimulating cell proliferation, increasing cell motility, and supporting tumor angiogenesis. Moreover, there is mounting evidence that autocrine production of prolactin plays a more significant role in tumor growth than pituitary prolactin production. This is evidenced by dopamine analogs failing to suppress prolactin production in sites outside the pituitary gland. Notably, bromocriptine-induced normalization of prolactin levels has not yielded beneficial outcomes in managing metastatic breast and prostate cancers. The molecular mechanisms underlying the altered expression of prolactin and its receptor in malignant thyroid nodules warrant further investigation (10).

The current study examined the association between thyroid peroxidase antibody (TPO-Ab) levels and thyroid nodules. The patients with nodules demonstrated a significantly elevated level of TPO-Ab compared to the group without nodules. Positive TPO-Ab status in individuals with nodules was correlated with higher thyroid-stimulating hormone levels and a higher incidence of ultrasound features indicative of malignancy. As posited by previous research, thyroid autoantibodies, such as TPO-Ab, could serve as a potential risk factor for malignancy in patients with thyroid nodules. Webmin Xu reported similar results in 2017 (15). The meta-analysis by Liu et al., 2020, evaluated the association between thyroid autoantibodies particularly thyroid peroxidase antibodies (TPO-Ab) and the risk of thyroid cancer from 19 observational studies involving a total of 31,958 participants. The analysis revealed that individuals with positive TPO-Ab had a significantly higher risk of developing thyroid cancer compared to those who were antibody-negative (16). The level of TSH hormone was significantly higher among patients with nodules than in the control group. Also, further investigations indicated a higher level of TSH in the nodule and TPO-Ab-positive group than in the nodule and TPO-Ab-negative group. Thyroid-stimulating hormone (TSH) is a thyroid cell growth factor, and the signaling pathway may be required to express other growth factors, receptors, and proto-oncogenes. Based on this theory, TSH suppression is an essential clinical therapeutic tool for managing thyroid cancer. Several studies have evaluated the role of TSH as a predictor of thyroid malignancy in recent years (17).

Since Boelart et al. reported a parallel increase in the risk of malignancy and serum TSH levels (18), several studies investigating the association between serum TSH levels and thyroid cancer have reported conflicting results (19-21). Although the present study did not confirm the malignancy of nodules by fine needle aspiration biopsy, comparing ultrasound features in TPO-Ab negative and TPO-Ab positive subgroups of patients with nodules suggests a higher prevalence of these predictive features in malignancies. Positive TPO-Ab in nodular patients was associated with a higher TSH level and solidity. These findings align with previous investigations by Lenara Golbert et al. (17), Fiore E et al. (19), and Haymart MR et al. (20), which demonstrated a positive correlation between higher TSH levels and malignant nodules. Further studies are needed to confirm these results and determine the clinical utility of TPO-Ab and TSH in diagnosing and managing thyroid nodules. The present study showed that the serum TSH, prolactin, and TPO antibody levels in patients with nodules are significantly higher than in those without. Also, positivity for TPO antibodies in patients with nodules is associated with a higher TSH level and solidity. As a result, the present study suggests higher TSH, prolactin, and TPO antibodies associated with thyroid nodules, especially solid nodules, and it may be an auxiliary factor in the prediction of malignant thyroid nodules.

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Authors' contribution: Maedeh Mataji provided the samples and contributed to data collection. Mehran Frouzania and Amirsaleh Abdollahi contributed to data collection and wrote the manuscript. Reza Ali Mohammadpour contributed to data analysis and revise the manuscript. Zahra Kashi supervised the work and revised the manuscript. Adele Bahar designed and conducted the research and revised the manuscript. All authors read and approved the final manuscript.

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