Original Research Article

The Egyptian Journal of Immunology

The Egyptian Journal of Immunology
Volume 29 (4), 2022: 156–162.
www.Ejimmunology.org

Serum IgG4 level for malignancy prediction in indeterminate thyroid nodules among patients with or without autoimmune thyroid disease

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Abstract

The incidence of thyroid nodules (TNs) has increased nowadays, and it is critical to properly differentiate between malignant and benign nodules to prevent unneeded thyroidectomy as well as complications related to surgery. IgG4 significantly contributes to various cancer-associated processes. The present study aimed to assess the value of serum IgG4 level in predicting malignancies in indeterminate thyroid nodules (ITN) among patients with and without autoimmune thyroid disease (AITD). A total of 67 patients with indeterminate cytology thyroid nodules (Bethesda III and IV, according to Bethesda system for reporting thyroid cytopathology) were selected. Preoperative serum thyroid profile, IgG4, anti-thyroglobulin (TG) and anti-thyroid peroxidase (TPO) antibody levels were determined. After total thyroidectomy, patients were categorized based on the postoperative histopathology outcome into two groups. Group (I): confirmed benign nodules (n=55) and Group (II): confirmed malignant TNs (n=12). IgG4 levels were significantly elevated among malignant TNs patients than in benign TNs patients, with a median (IQR) of 194.5 mg/dl (183 – 214) vs. 91 mg/dl (60 – 113), respectively (*P*=0.001). The cut-off value for differentiation between malignant and benign TNs was >180 mg/dl with a sensitivity of 75% and specificity of 100%. There was a significant positive correlation between thyroid antibodies and IgG4 levels (*P*=0.001). AITD patients had significantly higher level of IgG4 compared with those without AITD 189 mg/dl (153 – 208) vs 89 mg/dl (58 – 112), respectively (*P* =0.001). Eighty percent (12/15) of patients with AITD had malignant TNs with IgG4 >180 mg/dl, while 20% (3/15) of patients with benign TNs showed IgG4 levels <180 mg/dl. In conclusion, IgG4 level can be proposed as a predictor of malignant TNs.

Keywords: IgG4, ITN, Autoimmune thyroid antibodies, Thyroid malignancy, Bethesda III and IV.

Date received: 23 April 2022; **accepted:** 26 July 2022

Introduction

Despite the increased prevalence of thyroid nodules (TNs), only 5-10% are malignant and require surgical intervention.[1] The primary concern is to preoperatively differentiate the benign from the malignant lesions. For this

purpose, fine-needle aspiration cytology (FNAC) for TNs is the gold standard test due to its reliability, rapidness, safety, and cost-effectiveness. However, the non-diagnostic results requiring re-biopsy and the indeterminate cytology defined as the "gray zone" are the limitations of this method.[2] TNs are often correlated with increased autoimmune thyroid disease (AITD), especially Hashimoto's thyroiditis.[3] Thyroid autoimmunity, as well as thyroid cancer, are regarded as opposing ends of the immunological response.[4] In addition, chronic inflammation can be involved in tumorigenesis. Immunoglobulin G4-related disease (IgG4-RD) is a novel category that involves multiple organ systems, generally the endocrine system and particularly the thyroid. It is defined as a fibro-inflammatory disease with unique histological features and frequently elevated serum IgG4 levels, which are helpful in the diagnosis.[5] A study found that when the cut-off for serum IgG4 was established at higher than 135 mg/dL, the specificity and sensitivity to diagnose IgG4-RD were 60% and 90%, respectively, and that serum IgG4 measurement would be helpful in monitoring therapy response as well as recurrence.[6] Malignancies emerged in 10.4 percent of IgG4-RD individuals, which is approximately 3.5 times greater than the general population cancer occurrence.[7] Therefore, the present study aimed to determine the contribution of serum IgG4 level in predicting malignancy in cases with indeterminate thyroid nodules (ITN) among patients with and without AITD.

Subjects and Methods

Study population

A cross-sectional design was performed on 67 euthyroid subjects aged ≥ 18. The number of cases was estimated utilizing the 3rd version of the Power of Sample Size Calculation Program. The mean difference between malignant and benign cases was 69.2 power 80% and alpha error 0.05. From October 2019 to September 2021 cases were enrolled from Ain Sham University Hospitals (Endocrine Outpatient Clinic as well as the Endocrine Surgical Ward). They included patients with ITN; Thyroid Imaging Reporting and Data System (TR-IADS) score ≥ 3 by ultrasound (US) and Bethesda classification III and IV (according to the Bethesda system for reporting thyroid cytopathology)[1,2] by FNAC. Preoperative serum thyroid profile, IgG4 levels, anti-thyroid peroxidase (anti-TPO) levels, and anti-thyroglobulin (anti-TG) levels were evaluated. Patients underwent total thyroidectomy and were categorized based on the postoperative histopathology outcome into two groups. Group (I): cases with benign TNs (n=55), and Group (II): cases with malignant TNs (n=12). The individual histopathology of malignant TNs was as follows: one case with Hurthle cell neoplasm with encapsulated medullary micro- carcinoma, one case with anaplastic on top of papillary carcinoma, two cases with an invasive follicular variant of papillary carcinoma, and eight cases with papillary cell carcinoma. Patients with diseases affecting the level of serum IgG4, such as chronic liver disease, chronic kidney disease, and malignancies, were excluded from the study.

Ethical consideration

The Research Ethical Committee at the Faculty of Medicine, Ain Shams University reviewed and approved the protocol of the present study (MD 286 /2019). All the study subjects provided informed written consents.

Clinical and Biochemical analyses

All cases underwent detailed history taking, such as demographic data (sex, age) as well as full clinical history taking, including previous neck irradiation, any thyroid disease, family history of thyroid disease, or thyroid malignancy. A thorough physical examination included vital data, neck examination, and lymph node examination. A blood sample (5 mls) was aseptically withdrawn from each study participant. Blood samples were used for measuring free thyroxine (FT4), free triiodothyronine (FT3), and thyroid-stimulating hormone (TSH) using ELISA kits (manufactured by BioVendor, North Carolina, USA), according to the manufacturer's instructions. Anti-TG and anti-TPO antibodies were assessed by (a chemiluminescent

microparticle immunoassay kits (COBAS Elecsys® kit provided by Roche Diagnostics, Switzerland), according to the manufacturer's instructions. Serum IgG4 antibodies were measured with Immunoturbidimetric assay kits (Tina-quant IgG Gen kits provided by Roche Diagnostics, Switzerland), according to the manufacturer's instructions. Precision was determined using Elecsys reagents and pooled human sera according to a modified protocol of the Evaluation Precision version 5A (EP5A) of the CLSI (Clinical and Laboratory Standards Institute). Analysis of the samples was performed utilizing a COBAS E601 fully automated analyzer (Roche Diagnostics International, Rotkreuz, Switzerland). Patients with AITD was identified by serum anti-TPO antibodies >30 (IU/ml) and/or serum anti-TG antibodies >85 (IU/ml).

Ultrasonography of thyroid gland

Thyroid gland US was performed for all patients at Ain Shams University hospital to assess thyroid parenchyma. In addition, data were analyzed as well as calculated based on the American College of Radiology Thyroid Imaging Reporting & Data System (ACR-TI-RADS) scoring rubric for malignant risks.[8] It should be noted that the research included suspicious lesions with TI-RADS scores ≥ 3.

Fine needle aspiration biopsy and cytology (FNAC)

A specialized radiologist performed FNAC in accordance with Werga et al., 2000.[9] All samples were evaluated by specialized cytopathologists. Results were reported using the Bethesda system classification.[10] Suspicious lesions with TI-RADs scores 3 and 4 were included in this study and referred for FNAC.[10] Then, the patients were referred for surgery for total thyroidectomy at the Endocrine Surgical Department of Ain Shams University Hospital.

Histopathological examination

A specialist pathologist performed a histopathological examination of thyroidectomy specimens after the operation to detect patients with thyroid cancer.

Statistical methods

Data analysis was performed utilizing the 23rd version of IBM SPSS Statistics. Data were described as percentages or numbers for categorical variables. Regarding continuous variables, normal distribution data were described as means ± standard deviations (SD), while those with skewed distributions were described as medians with interquartile ranges (IQRs). For continuous data with satisfied normality, a t-test was utilized for comparison between groups; otherwise, a non- parametric test (Mann-Whitney) was utilized. Moreover, the Chi-square test was utilized for the comparison of categorical variables. Receiver operating characteristic (ROC) curve analysis was utilized to estimate the area under the curve (AUC) for thyroid malignancy predictors and find the best cut-off points. The statistical significance was determined at a *P*-value of less than 0.05.

Results

Analytical data of the study population are depicted in (Table 1).

Ultrasound findings among the studied population

There was a significantly higher prevalence of malignancy among cases with TIRADS IV 11/12 (91.7 %) compared to 1/12 (8.3%) in cases with TIRADS III, (*P*<0.001).

Fine needle aspiration cytology results

Of the 54 patients with Bethesda III nodules, one patient (1/54) had malignant histopathology indicating a 1.85% risk of malignancy. While from the 13 patients with Bethesda IV nodules, 11 patients (11/13) had malignant histopathology indicating 84.6% risk of malignancy.

IgG4 levels were significantly elevated among patients with malignant TNs (group II) compared to patients with benign TNs (group I) with median (IQR) 194.5 mg/dl (183 – 214) vs. 91 mg/dl (60 – 113), (*P*=0.001).

The Roc curve was used to differentiate between benign and malignant cases. At a cut-off value of >180 mg/dl the positive predictive

value (PPV) was 100, with negative predictive value (NPV) of 94.8%, specificity of 100%, and sensitivity of 75% (Table 2).

Serum IgG4 levels showed a statistically significant positive correlation between thyroid antibodies, anti-TG and anti-TPO (0.466 and 0.616, respectively) (*P*< 0.001) (Figures 1 and 2). IgG4 levels showed a statistically insignificant correlation with age (-0.015), TSH (-0.197), FT3 (-0.038), FT4 (- 0.165) (*P* >0.05 for all).

Autoimmunity, IgG4, and histopathology outcome of the studied population

Of the 67 studied patients, 15 (22.3%) patients had AITD, while 52 (77.6%) patients did not have AITD. In addition, AITD was substantially more prevalent among patients in Group II compared to Group I. Patients with AITD had significantly higher level of IgG4 with median (IQR) 189 mg/dl (153 – 208) compared to those without AITD with 89 mg/dl (58 – 112), (*P*< 0.001). Of the 15 patients with AITD 12 (80%) patients had malignant TNs with IgG4 >180 mg/dl, while the 3 patients (20%) with benign TNs showed IgG4 levels <180 mg/dl.

Table 1. Analytical data of the studied subjects.

	Pathology		*P*- value
	Benign (Group I) n= 55	Malignant (Group II) n= 12	
Age (years) Mean±SD	43.84 ± 11.45	38 ± 10.90	NS
Sex			
Males n= (%)	0 (0%)	2 (16.6%)	0.002
Females n= (%)	55 (100%)	10 (83.3%)	
Personal history	No previous history of cancer or neck irradiation		
Family history	Negative family history of cancer or cancer thyroid		
TSH (μIU/ml) Median (IQR)	0.9(0.75–1.06)	3.09 (2.59 – 3.64)	NS
FT3 (pg/ml) Mean±SD	3.08 ± 0.66	2.93 ± 0.27	NS
FT4 (ng/dl) Mean±SD	1.19 ± 0.27	1.14 ± 0.25	NS
Anti-TPO (IU/ml Median (IQR))	6.14(4.3–9.04)	55.67(40.39–67.89)	0.001
Anti-TG (IU/ml) Median (IQR)	18.98 (10.8 –33.5)	309.5(145–405.5)	0.001
IgG4 (mg/dl) Median (IQR)	91 (60 – 113)	194.5 (183 – 214)	0.001

P > 0.05 is not significant (NS); Independent t-test;: Chi-square test. ≠: Mann-Whitney test; IQR: Inter-quartile range; TSH: thyroid stimulating hormone; FT3:triiodothyroinin; FT4:thyroixin; anti TPO: anti-peroxidase antibodies;
anti-TG: anti- thyroglobulin antibodies.

Table 2. The cut-off point to differentiate between benign TNs (group I) and malignant TNs (group II) regarding the IgG4 level.

Cut off point	AUC	Sensitivity	Specificity	+PV	-PV
> 180 (mg/dl)	0.875	75.00	100.00	100	94.8

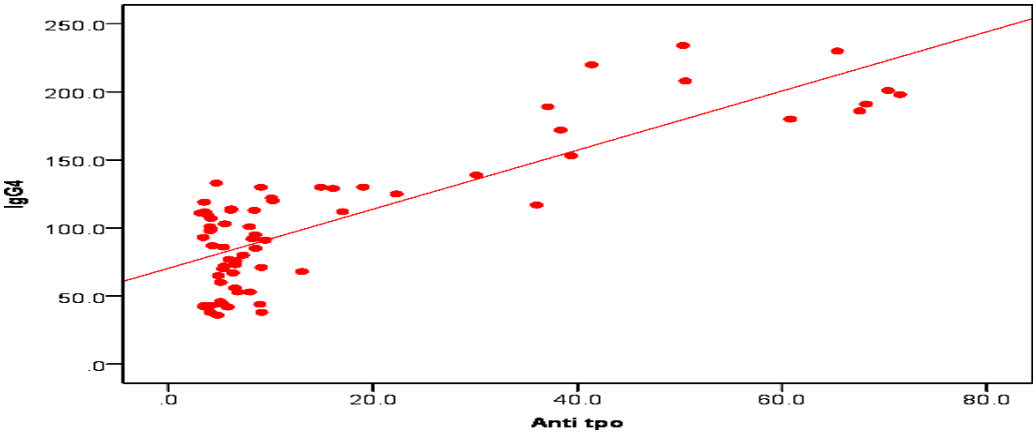

Figure 1. Correlation of IgG4 levels with anti-TPO antibody levels.

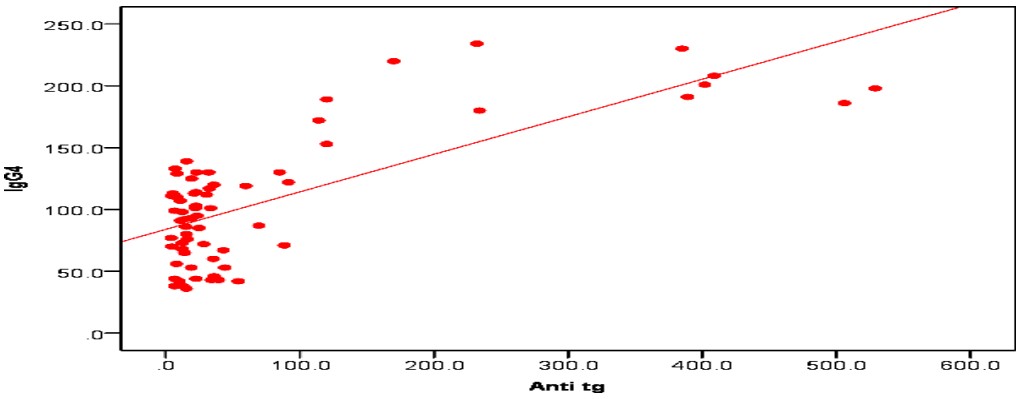

Figure 2. Correlation of IgG4 levels with anti-TG antibody levels.

Discussion

The present study aimed to determine the contribution of serum IgG4 level in predicting malignancy in cases with indeterminate thyroid nodules (ITN) among patients with and without AITD. Obtained data revealed significantly higher serum IgG4 levels among malignant TNs patients (Group II) compared to benign TNs cases (Group I). In addition, all the patients in Group II (n=12) versus only three cases in Group I had AITD with a statistically significant difference.

Our study showed that all the patients with malignant TNs versus none of the those with benign TNs had a serum IgG4 level above 180 mg/dl which was found to be the cut-off value for predicting malignant TNs with 75% sensitivity and 100% specificity. These findings agreed with those of a study by Taşli et al., 2014 who showed that malignancy was detected in 76% of cases with elevated IgG4 level.[11] Consequently, IgG4 association with malignancies was statistically significant.[11] A study by Hubers, et al., 2018 illustrated that IgG4 is hypothesized to impair anti-tumor immunity via suppressing the activation of immune effector cells with cancer cells directly causing increased IgG4 via the agency of IL-10-related cytokines.[12] In addition, our study confirmed that an elevated serum level of IgG4 >180 mg/dl is an independent predictor of thyroid carcinoma.

The link between AITD and thyroid cancer is debatable.[13] Our data revealed that AITD was associated with an elevated thyroid carcinoma risk. This was compatible with findings of a study by Wong et al., 2015 who detected a statistically significant association between AITD disease and malignancy.[13] Also, a study by Seifman et al., 2011 reported that the malignancy rates were more prevalent in AITD patients than non-AITD (23.2% versus 11.4%, $P < 0.001$).[14] In contrast, other studies revealed that

the presence of thyroiditis in TNs subjects does not increase malignancy risks.[6] The study by Anil et al., 2010 reported malignancy rates similar in TNs cases with and without AITD (1.0% and 2.7%, respectively).[15] These differences between studies may be attributed to that not all the studied patients underwent thyroidectomy for their TNs. Hence, malignant TNs might have gone undetected. Indeed, the diagnosis of malignancy based on a post-thyroidectomy histopathological report would support our findings.

IgG4-related thyroiditis is a novel clinicopathologic entity marked by thyroid inflammation rich in substantial fibrosis as well as IgG4-positive plasma cells.[16] IgG4-positive thyroiditis is clinically associated with a higher level of circulating thyroid antibodies.[17] Similarly, we found a positive correlation between IgG4 levels with anti-TPO levels and anti-TG levels.

There are several TNs risk factors, including age, sex, family history of thyroid cancer, and history of neck irradiation.[18] In the current study, there was no significant difference in age between patients with benign nodules and those with malignant nodules. Also, a study by Witczak et al., 2016 reported no difference in age between benign TNs cases and malignant TNs cases.[19] On the contrary, a study by Boonrod et al., 2021 illustrated that patient age was a predictor of malignancy; nonetheless, their study addressed follicular and Hurthle cell neoplasms and studied a cut-off age of prediction of malignancy > 55 years.[20]

Thyrotropin concentration as an indicator of TNs.[21] In the current study, the concentration of serum TSH did not differ between cases with malignant and benign TNs. This is consistent with findings of a study by Kim et al., 2011 who reported that TSH concentration was not different among patients with benign disease or PTC.[22] In conclusion, our data showed that serum IgG4 levels were substantially elevated among malignant TNs patients than those with benign TNs at a cut-off value above 180 mg/dl. This may indicate that IgG4 levels could be proposed as a predictor of malignant nodules among patients with and without AITD disease.

Acknowledgements

The authors would like to express their great thanks to the Endocrine Clinic staff, the Endocrinology Department staff, the General Surgery Department Staff, the Radiology staff, the Pathology team, and all the patients who participated in this study.

Author Contributions

All authors shared in the design of this study. MRH, IZA and RKE proposed the idea. Data collection and sampling was done by RKE. Data analysis and interpretation were done by NFA, AMB, NRM and RKE. Writing and revision of the manuscript were done by MRH, IZA, AMB, NRM and RKE. All authors read and approved the final manuscript.

Declaration of Conflicting Interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Funding

The author(s) denies receipt of any financial support for the research, authorship, and/or publication of this article.

Ethical approval

The Research Ethical Committee of the Faculty of Medicine, Ain Shams University reviewed and approved the study protocol (MD 286 /2019).

Informed consent

An informed written consent was obtained from each participant in our study.

References

1. Arul P., Akshatha C., Masilamani S. (2015). A study of malignancy rates in different diagnostic categories of the Bethesda system for reporting thyroid cytopathology: An institutional experience. *Biomed J;* 38(6):517–22.
2. Bongiovanni M., Spitale A., Faquin W.C., Mazzucchelli L., Baloch Z.W. (2012). The Bethesda system for reporting thyroid cytopathology: A meta-analysis. *Acta Cytol;* 56(4):333–9.
3. Ruggeri R.M., Campennì A., Sindoni A., Baldari S., Trimarchi F., Benvenga S. (2011). Association of autonomously functioning thyroid nodules

with Hashimoto's thyroiditis: Study on a large series of patients. *Exp Clin Endocrinol Diabetes*; 119(10):621–7.

4. Yamamoto M., Takahashi H., Tabeya T., et al. (2012). Risk of malignancies in IgG4-related disease. *Mod Rheumatol*; 22(3):414–8.
5. Tabata T., Kamisawa T., Takuma K., et al. (2011). Serial changes of elevated serum IgG4 levels in IgG4-related systemic disease. *Intern Med;* 50(2):69–75.
6. Yu Y., Zhang J., Lu G., et al. (2016). Clinical relationship between IgG4-positive Hashimoto's thyroiditis and papillary thyroid carcinoma. *J Clin Endocrinol Metab;* 101(4):1516–24.
7. Ito M., Naruke Y., Mihara Y., et al. (2011). Thyroid papillary carcinoma with solid sclerosing change in IgG4-related sclerosing disease. *Pathol Int;* 61(10):589–92.
8. Tessler F.N., Middleton W.D., Grant E.G. (2018). Thyroid imaging reporting and data system (TI-RADS): A user's guide. *Radiology*; 287(1):29–36.
9. Werga P., Wallin G., Skoog L., Hamberger B. (2000). Expanding role of fine-needle aspiration cytology in thyroid diagnosis and management. *World J Surg*; 24(8):907–12.
10. Cibas E.S., Ali S.Z. (2017). The 2017 Bethesda System for Reporting Thyroid Cytopathology. Thyroid;27(11):1341–6.
11. Taşli F., Özkök G., Argon A., et al. (2014). The role of IgG4 (+) plasma cells in the association of Hashimoto's thyroiditis with papillary carcinoma. *APMIS*; 122(12):1259–65.
12. Hubers L.M., Vos H., Schuurman A.R., et al. (2018). Annexin A11 is targeted by IgG4 and IgG1 autoantibodies in IgG4-related disease. *Gut*; 67(4):728–35.
13. Wong S.L., Grodski S., Yeung M.J., et al. (2015). Anti-thyroid antibodies as a predictor of thyroid cancer. *ANZ J Surg*; 85(11):849–53.
14. Seifman M.A., Grodski S.F., Bailey M., Yeung M.J., Serpell J.W. (2011). Surgery in the setting of Hashimoto's thyroiditis. ANZ J Surg; 81(7–8):519–23.
15. Anil C., Goksel S., Gursoy A. (2010). Hashimoto's thyroiditis is not associated with increased risk of thyroid cancer in patients with thyroid nodules: A single-center prospective study. *Thyroid*; 20(6):601–6.
16. Li Y., Wang X., Liu Z., et al. (2020). Hashimoto's Thyroiditis with Increased IgG4-Positive Plasma Cells: Using Thyroid-Specific Diagnostic Criteria May Identify Early Phase IgG4 Thyroiditis. *Thyroid*; 30(2):251–61.
17. Lintusaari J., Vesaniemi E., Kalfert D. et al. (2020). IgG4- positive plasma cells in Hashimoto thyroiditis: IgG4-related disease or inflammation-related IgG4-positivity? *APMIS*; 128(9):531–8.
18. Crnčić T.B., Tomaš M.I., Girotto N. et al. (2020). Risk factors for thyroid cancer: What do we know so far? *Acta Clin Croat*; 59(1):66–72.
19. Witczak J., Taylor P., Chai J., et al. (2016). Predicting malignancy in thyroid nodules: Feasibility of a predictive model integrating clinical, biochemical, and ultrasound characteristics. *Thyroid Res*; 9 (1):1–7.
20. Boonrod A., Akkus Z., Castro M.R., et al. (2021). Thyroid Nodule Size as a Predictor of Malignancy in Follicular and Hurthle Neoplasms. *Asian Pacific J Cancer Prev*; 22(8):2597– 602.
21. Choi J.S., Nam C.M., Kim E.K. et al. (2015). Evaluation of serum thyroid-stimulating hormone as indicator for fine-needle aspiration in patients with thyroid nodules. *Head Neck*; 37(4):498–504.
22. Kim K.W., Park Y.J., Kim E.H., et al. (2011). Elevated risk of papillary thyroid cancer in Korean patients with Hashimoto's thyroiditis. *Head Neck*; 33(5):691–5.