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Histophysiology study of interleukin-4 in thyroid cancer patients

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Introduction. Interleukins have promising prospects in the clinical treatment of cancer. Interleukin-4 (IL-4) is an anti-inflammatory cytokine with an immunosuppressive effect on antitumor activity by immune cells, but the mechanical action of IL-4 in thyroid cancer is unknown. Aim: to investigate the effect of IL-4 expression in thyroid cancer patients. Furthermore, to clarify the association between obesity and thyroid cancer.

Material and methods. The present study was conducted on 115 subjects with thyroid nodules (36 with thyroid cancer and 79 with benign lesions) in Basrah, Iraq, from November 2019 to April 2022. To conduct a histophysiology study of IL-4.

Results. There was a significant difference in serum IL-4 between the thyroid cancer and control subjects. A higher level of serum IL-4 was observed in the Hashimoto thyroiditis group. There was no significant difference in body mass index (BMI) between thyroid cancer and control subjects. The expression of tissue IL-4 in thyroid cancer patients was strong in 8 (22.22%) slides, moderate in 7 slides (19.44%), weak in 8 slides (22.22%), and negative in 13 slides (36.11%), while in the control group, it was strong in 7 (30.44%) slides, moderate in 8 slides (34.79%), weak in 5 slides (21.74%) and negative in 3 slides (13.03%).

Conclusions. These findings indicate that serum levels of IL-4 may help diagnose thyroid cancer and identify patients with active disease who deserve closer medical attention. Furthermore, the secretion of IL-4 was systematic and not localized in thyroid cancer tissues. Obesity was not associated with a prevalence of thyroid cancer.

Key words: thyroid cancer, IL-4, obesity, thyroid gland, histophysiology

Introduction

Cancer is a significant public health problem worldwide [1]. Cancer is a class of disease characterized by the uncontrolled division of cells and the ability of these cells to invade other tissues, either by direct invasion into adjacent tissue or by implantation into distant sites (metastasis) [2, 3]. Thyroid cancer is the most dominant cancer type of the endocrine system [4]; its prevalence has increased dramatically worldwide in recent decades [4–7] as a result of environmental factors, radiation exposure, and the rapid development of available imaging and tools used for the detection of thyroid nodules [7–9]. Thyroid cancer accounts for approximately 2.3% of all new cancer cases in the U.S. [4]. Furthermore, it accounts for ≤1% of all human malignancies, a relatively rare disease responsible for

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six deaths per million annually [10]. Thyroid nodules represent the majority of lesions found in 19–68% of randomly selected people, and most benign nodules are without complications [10]. Seven percent of them may have a suspicious nodule for thyroid cancer depending on age, sex, radiation exposure, family history, and other factors [5, 11].

In the Iraqi population, thyroid nodules are common. However, thyroid cancer accounts for 1.7% of these nodules [12], while Mansour et al. [13] found that the prevalence of thyroid cancer was 0.4% (No. = 77) from 17878 patients who presented with thyroid lesions in Basrah province.

Many studies have documented that the overall incidence of thyroid carcinoma has increased more rapidly than that of any other malignancy in recent years, especially in women [14], and many serum interleukins have been medically used as diagnostic and prognostic markers or treatments for various types of diseases especially malignant disease [15, 16]. IL-4 has an essential role in inhibiting growth in many kinds of human cancers, including renal and gastric carcinoma [17]. Although many studies demonstrated that IL-4 and IL-10 are anti-inflammatory cytokines that have the immunosuppressive effect of antitumor activity, allowing tumor cells to escape recognition and attack by the immune system which can lead to cancer cell proliferation and metastasis. The mechanism of action of IL-4 in thyroid cancer is unknown [18–20], so understanding the mechanisms of interleukins in thyroid cancer will provide new targets for immunotherapy of thyroid cancer or finding alternative tools to discriminate thyroid cancer from benign lesions. The overall goal of this work was to investigate the effect of IL-4 expression in the blood serum and tissues of thyroid cancer patients. Furthermore, it aims to clarify the association between obesity and thyroid cancer.

Materials and methods

The study population consisted of 36 patients with thyroid cancer (11 men, 25 women) and 79 with benign thyroid lesions (7 men, 72 women); the mean age of thyroid cancer samples was 36.166 ± 16.84 years, and the mean age of control samples was 40.016 ± 10.519 years. All subjects were undergoing health checkups in Iraq/Basrah province hospitals and medical centers from November 2019 to April 2022. For the immunohistochemistry (IHC) study of IL-4 expression in thyroid cancer patients, all blood samples were collected by collecting 5 ml of peripheral venous blood without anticoagulant and allowed to clot in gel tubes at room temperature to study IL-4 expression in thyroid cancer patients. The IL-4 ELISA kit (catalog No.: E-EL-H0101) by Elabsceince/China (USA brand) was used to determine human IL-4 in blood serum. BMI was determined according to [21].

Fifty-seven paraffin wax-embedded tissues were collected from patients after surgery for both thyroid cancer (n = 36) and benign (control) subjects (n = 23) and were divided into three categories, Graves' disease (n = 4), Hashimoto's disease (n = 4), and multinodular goiter (n = 15). Then, the samples were stored at 5–8ºC until use in the study.

For investigating IL-4 expression in tissues, the IL-4 primary antibody (catalog No.: E-AB-62102) from Elabsceince/China was used, and IHC staining was accomplished according to [22]. A semiquantitative method (Allred) was used to interpret IL-4 immunohistochemical staining [23].

The effect sample size of this was calculated depending on the Kish formula [24]:

$$n = \frac{Z^2\, p(p-1)}{d^2} = \frac{(1.96)^2\, \xi\, 0.02(0.02-1)}{(0.05)^2} = 30.11 \qquad [24]$$

Statistical analysis: SPSS software version 26 was used for data analysis, and the ANOVA table and *post hoc* general liner model (GLM) were used to test the significance between different means. The Pearson correlation and Chi-square were used to examine the association between category variables [25].

Results

The result showed that there was no significant difference ($p \leq 0.05$) in BMI between cancer patients and control subjects since the values were 25.383 ± 5.39 kg/m^2 and 26.819 ± 3.92 kg/m^2, respectively (fig. 1). At the same time, there was a significant difference ($p \leq 0.05$) in serum IL-4 (pg/ml) between thyroid cancer patients and control subjects, with the value of 360.693 ± 241.493 pg/ml and 278.609 ± 82.729 pg/ml, respectively (fig. 2).

During the comparison of the IL-4 (pg/ml) level among diagnosis categories, the results showed a significance difference ($p \leq 0.05$) between thyroid cancer and multinodular goiter (MNG), since the value was 342.788 ± 234 pg/ml and 269.126 ± 76.05 pg/ml respectively. A higher serum IL-4 pg/ml level was observed in the Hashimoto thyroiditis group (383.67 ± 119.01 pg/ml) (tab. I).

There was a significant positive correlation ($r = 0.75$, $p = 0.013$) between serum level IL-4 (pg/ml) in thyroid cancer patients and BMI (kg/m^2). In contrast, the results of the Pearson correlation analysis in benign samples showed a negative correlation between serum level IL-4 and BMI (kg/m^2) ($r = –0.035$, $p = 0.756$) (fig. 3, 4).

For the histological study, all thyroid tissues were divided into two major groups of thyroid cancer and benign thyroid lesions, the benign thyroid tissues were divided into three categories, Graves' disease (n = 4), Hashimoto's (n = 4), and multinodular goiter (n = 15).

Thyroid cancer

The examination of thyroid cancer slides shows that all 36 samples (11 men and 25 women) belonged to papillary thyroid carcinoma, characterized by typical distinctive features. The tumor area and the normal thyroid parenchyma consists of different size follicles surrounded by normal

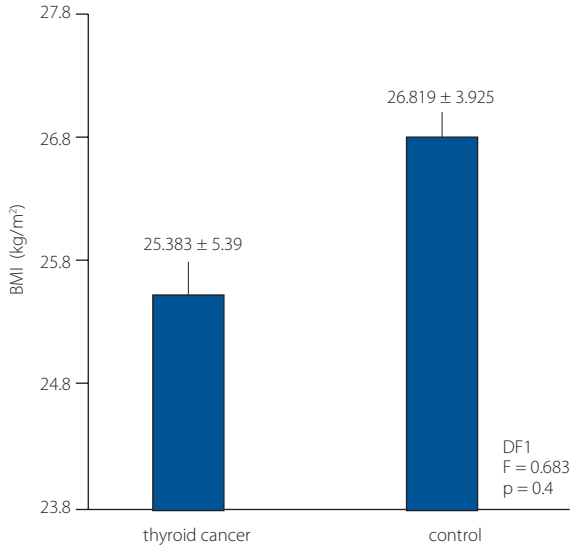

Figure 1. Distribution of BMI (kg/m^2) in thyroid cancer and control subjects

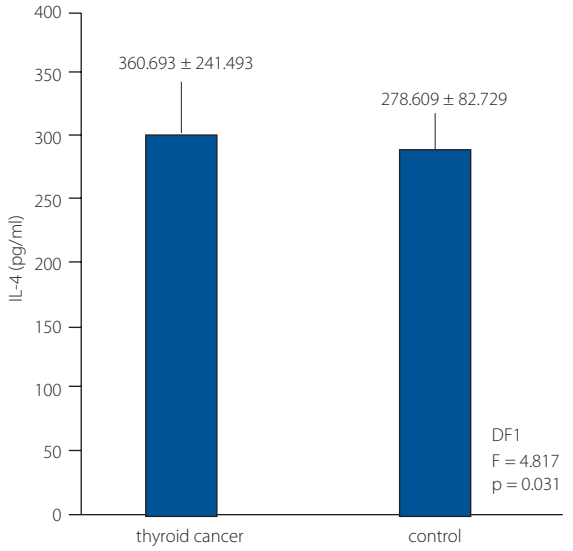

Figure 2. Level of serum IL-4 (pg/mL in thyroid cancer and control subjects

Table I. Level of IL-4 (pg/ml) in all subjects

Diagnosis		No.	Percent (%)	IL-4 (pg/ml) mean ± SD
cancer		25	24.03	342.788 ± 234[a]
control group	hyperthyroidism	9	8.66	310.195 ± 65.035[ab]
	hypothyroidism	2	1.93	324.082 ± 155.77[ab]
	Graves	10	9.61	262.839 ± 133.376[ab]
	MNG	54	51.92	269.126 ± 76.05[b]
	Hashimoto	4	3.84	383.67 ± 119.01[ab]

LSD – cancer × MNG = 73.66, p = 0.046*, cancer × hyperthyroidism, p = 0.523$^{N.S.}$, cancer × hypothyroidism, p = 0.822$^{N.S.}$, cancer × Graves, p = 0.338$^{N.S.}$, cancer × Hashimoto, p = 0.52$^{N.S.}$. The mean difference is significant at p ≤ 0.05. N.S – non-significant at level p ≤ 0.05

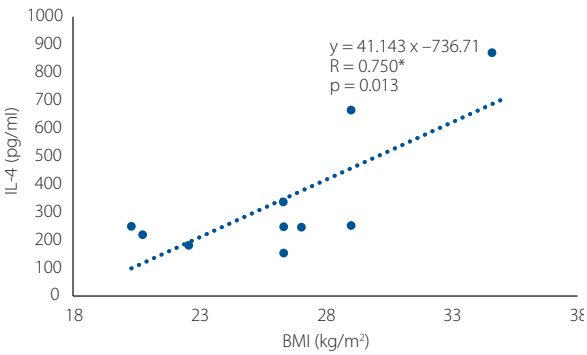

Figure 3. Correlation between IL-4 (pg/ml) level and BMI (kg/m^2) in thyroid cancer samples

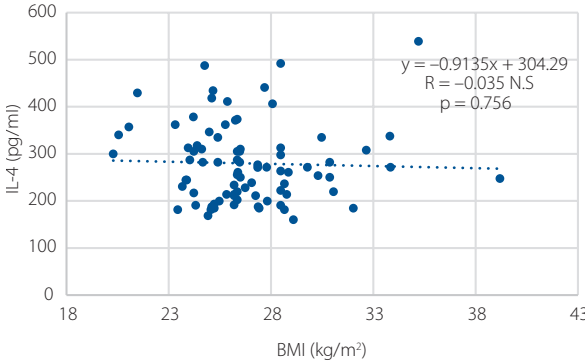

Figure 4. Correlation between IL-4 (pg/ml) level and BMI (kg/m^2) in control samples

thyroid cells filled with colloids separated by thin and thick capsules of collagen bundles, while the papillary tumor area is characterized by many papillary nuclear features, nuclear enlargement, nuclear clearing, and nuclear grooves, with multiple blood vessels (fig. 5).

In addition, another section of the papillary thyroid carcinoma shows papillary and follicular patterns, solid growth, and micro follicles separated by collagen fibers. During high power magnification, there were many sites of capsular and vesicular invasion, with papillary nuclear features such as nuclear clearing, nuclear grooves, and inclusion bodies, in addition to many sites of vascular and capsular invasion by malignant cells inside the vascular space of the tumor capsule (fig. 6).

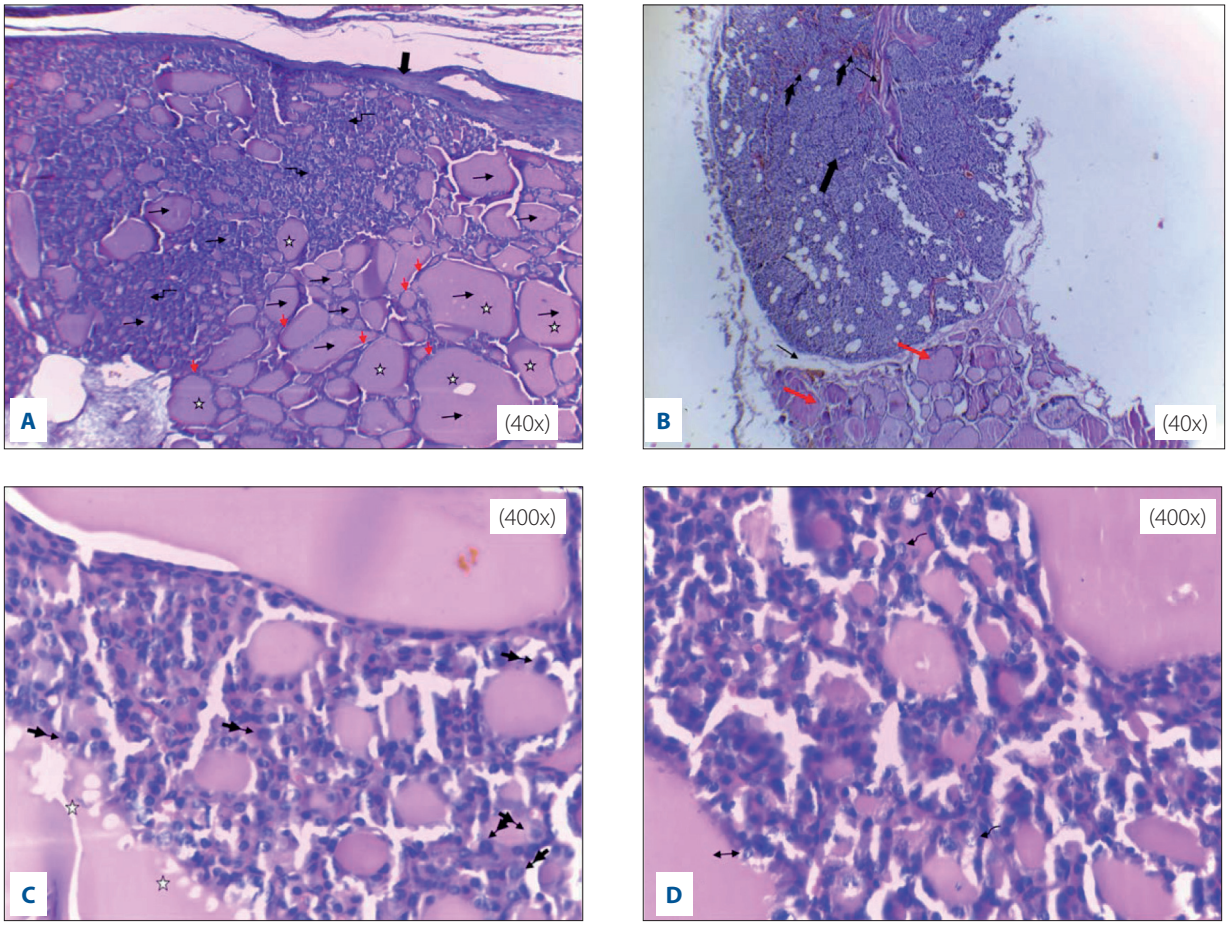

Figure 5. A section of the papillary thyroid carcinoma; (**A**) representative view showing a mixture of different size follicles black arrow (→) diffusely present papillary nuclear features cells (↱), follicles filled by colloid (pink color) (☆) and lined by normal-appearing cells (→), thick capsule (➡); (**B**) shows papillary nuclear feature cells (➡), follicle growth pattern (→), capsule of collagen fibers (→) blood vessels also presented (↱), (H&E); stain (40x). (**C**) and (**D**) section show enlarged and irregular nuclei (➡), nuclear groove (↔), and nuclear clearing (↱) with follicles growth pattern filled with colloid (pink color ☆) (H&E); stain 400x

Semiquantitative detection of IL-4 in thyroid gland tissues by immunohistochemistry assay

The expression of IL-4 in the thyroid tissues of cancer patients was strong in 8 (22.22%) slides (total 36 slides), moderate in 7 (19.44%), weak in 8 (22.22%), and negative in 13 (36.11%), with no significant difference $p \leq 0.05$ between the two groups (cancer and control) (Chi-square 5.345, $p = 0.148$) (tab. II and fig. 7).

The expression of IL-4 in the control group was strong in 7 slides (30.44%), moderate in 8 slides (34.79%), weak in 5 slides (21.74%), and negative in 3 slides (13.03%) (tab. II and fig. 8).

Discussion

Interleukins are immunoregulatory proteins secreted in response to several stimuli and play a vital role in cancer diseases as initiation, progression, and elimination [16]. IL-4 is an anti-inflammatory cytokine that regulates the immune response in normal health conditions and under cancers [26]. The present study demonstrates a significant difference in level of IL-4 in thyroid cancer patients than both control subjects and MNG groups, and these findings agree with Zivancevic-Simonovic et al. [27], who found that IL-4 level was higher in thyroid cancer patients than in control subjects. IL-4 is a potent immunosuppressive cytokine that has an important role in maintaining and proliferating cancer cells and helping them to escape from the immune system [20]. Safi et al. [28] found that a high level of IL-4 was associated with the reoccurrence of lung cancer, and Todaro et al. [29] found that IL-4 is required for the survival and growth of thyroid cancer cells. Although thyroid cancer cells do not constitutively produce IL-4, our results support a thyroid cancer induce infiltrating cells to produce IL-4.

Z. Li et al. [30] suggested that endogenous IL-4, the product of host immune response, can be used by tumor cells to facilitate their growth. IL-4 might act as a pro tumoral agent [31]. On the other hand, IL-4 may have an antitumor role since it acts synergistically with interferon-c to prime maturing antigen-presenting dendritic cells to produce high levels of a Th1 cytokine IL-12 that induces the differentiation of tumor-specific Th1-cells and cytotoxic T lymphocytes [32]. In contrast, previous studies indicate that although genetic variants in IL-4 do not affect the risk or outcome of differentiated thyroid cancer (DTC) patients, their influence on

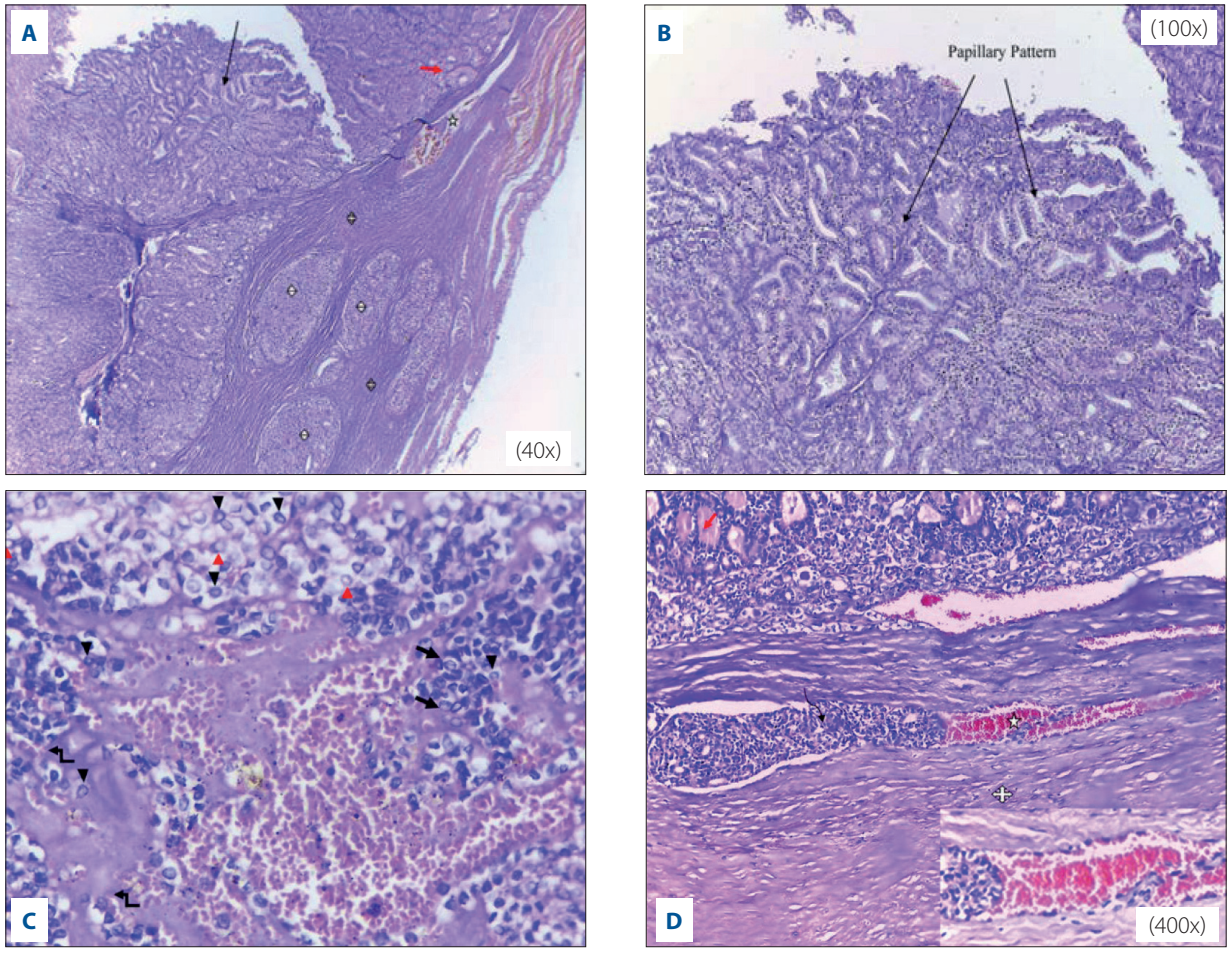

Figure 6. Papillary thyroid carcinoma; (**A**) showing a papillary pattern (⟶) with solid growth pattern (◈) and micro follicle pattern (⟶) separated by prominent collagen fibrosis tissue (✥) H&E; stain 40x. (**B**) in high magnification view from the same section, H&E; stain 100x. (**C**) shows many sites of capsular invasion (↰), nuclear clearing (▲), nuclear grooves (↘), and inclusion body (▼) (H&E); stain 400x. (**D**) shows vascular invasion (↷) inside the vascular space (☆) of the tumor capsule, H&E stain; (100x) and a high-power picture in the left corner; stain 400x

Table II. The immunohistochemistry score of IL-4 in thyroid cancer and control tissues

Diagnosis		Immunohistology score						
		negative	week	moderate	strong	total	person Chi-square	p value
malignant	count	13	8	7	8	36	5.345	0.148$^{N.S.}$
	%	36.11%	22.22%	19.44%	22.22%	100%		
benign	count	3	5	8	7	23		
	%	13.03%	21.74%	34.79%	30.44%	100%		

N.S. – non-significant at level $p \leq 0.05$

the behavior of thyroid tumors deserves further investigation [31]. Many studies reported a direct inhibitory effect of IL-4 on the growth of human gastric cancer, melanomas, spontaneous adenocarcinoma, fibrosarcoma, and renal cell carcinoma [17, 33–35].

The higher production of serum IL-4 in the present study was observed in the Hashimoto thyroiditis group. Moreover, Hashimoto's is an autoimmune disease characterized by infiltrating lymphocytes inside thyroid tissue [36]. Many studies have demonstrated that significant amounts of IL-4 are secreted by T cells, helper T lymphocyte type 2 (Th2), mast cells, eosinophils, and basophils [20, 37]. The high level of IL-4 in the Hashimoto thyroiditis group in our study was in response to the increasing number of lymph cells which have an essential role in the secretion of IL-4. Our results are in agreement with Zivancevic-Simonovic et al. [27] and Schuetz et al. [38] since they have also found increased IL-4 production in patients with Hashimoto thyroiditis.

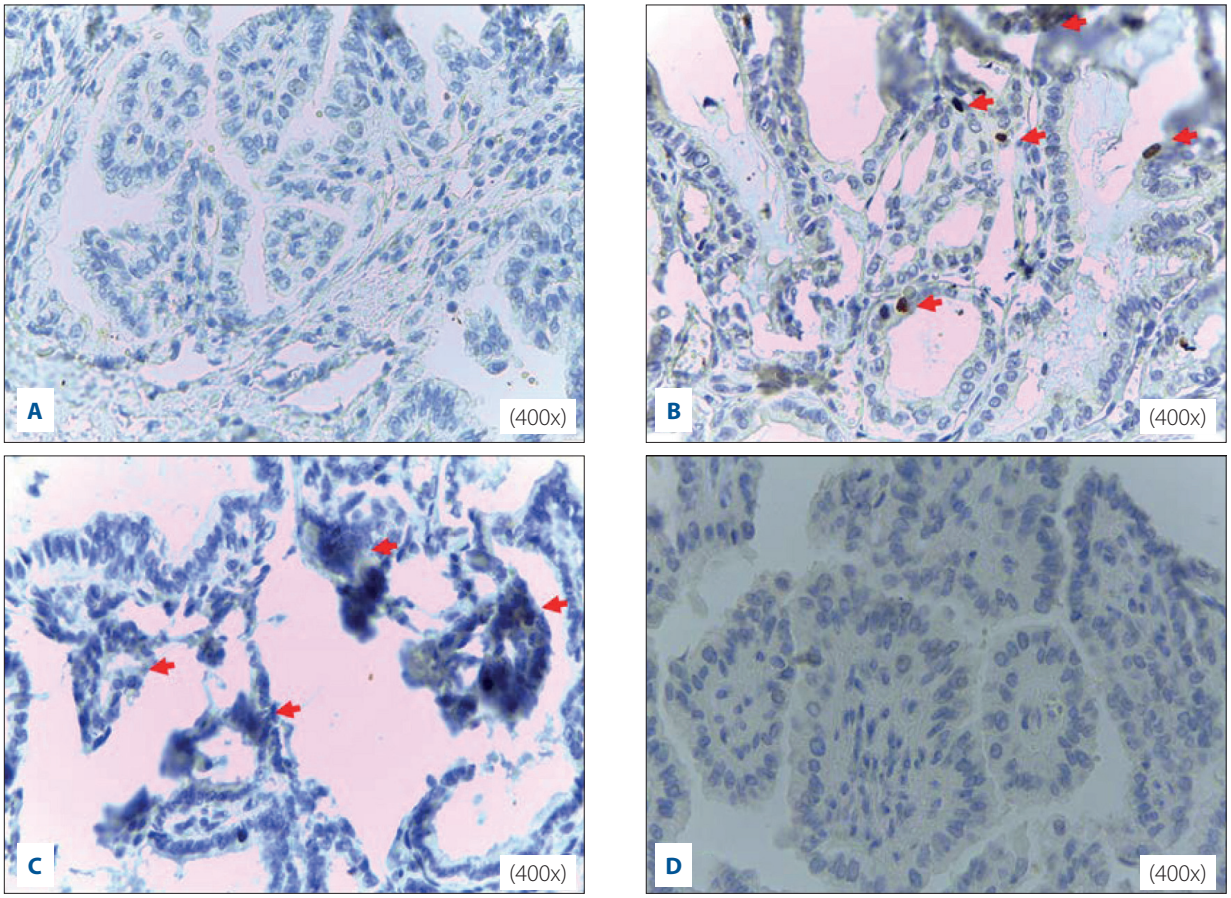

Figure 7. Immunohistochemical expression of IL-4 in thyroid cancer tissues; (**A**) section showing a negative expression; (**B**) weak positive staining, a red arrow (⟶); (**C**) strong positive staining, (red arrow); (**D**) negative control of thyroid cancer tissue; stain 400x

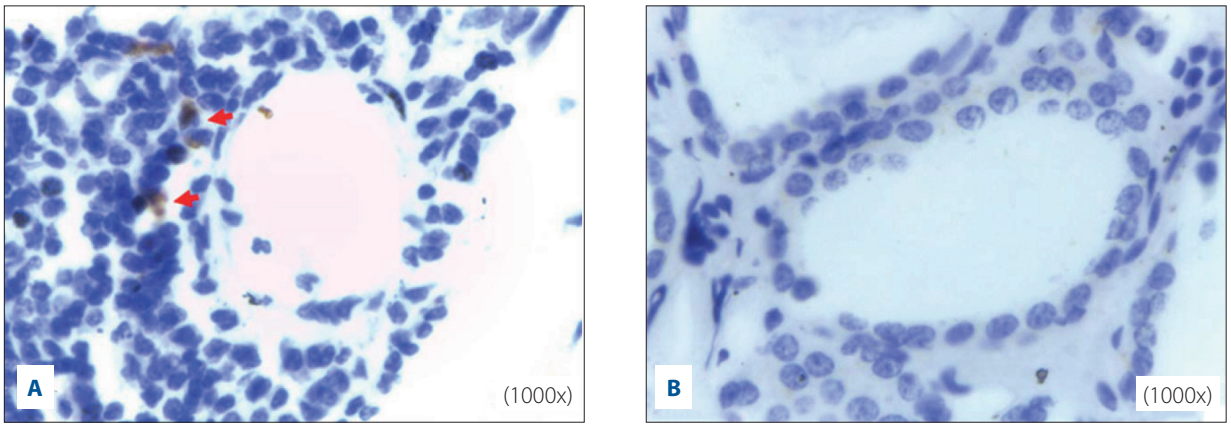

Figure 8. Immunohistochemical expression of IL-4 in benign thyroid tissues; (**A**) positive staining, red arrow (⟶); (**B**) negative control of benign thyroid tissue; stain 1000x

Because thyroid cancer is a rare disease and accounts for less than 1% of all cancer types in the human body [10], and the majority of thyroid cancer is papillary carcinoma [39], our study supports this finding (that the majority of thyroid cancer is papillary carcinoma) due to all the cancer samples belonging to papillary thyroid carcinoma, is the most prevalent type of thyroid cancer [40, 41], but we did not record any other thyroid cancer type due to its rare prevalence.

The present study confirms that obesity was not associated with a prevalence of thyroid cancer, there was no significant difference in BMI between cancer patients and control subjects.

Obesity has become a widely prevalent global health problem [42]. It has been posited that obesity causes thyroid cancer [43–45]. Furthermore, a correlation between being overweight and thyroid cancer is not widely accepted. A retrospective study of 4849 patients with thyroid nodules

(3809 females and 1040 males) did not confirm the positive correlation between thyroid cancer and obesity [46]. A similar conclusion has been reported by Ramdass et al. [47], which concluded that there was no correlation between BMI and development of thyroid cancer clinicopathological features [48].

In a histological study of IL-4, the current study revealed that the tissue expression of interleukin did not correlate with serum interleukin levels. A similar conclusion was reached by [49]. The results of IL-4 expression in the current study revealed no significant difference between thyroid cancer and the control groups. The expression of IL-4 was similar in both the control and thyroid cancer tissues. These findings are in agreement with de Oliveira et al. [50] which found that IL-4 regulates the immune system response, the expression of IL-4 in tissues is not engaged in the clinicopathology characteristics of cancer. However, many studies have investigated that IL-4 expression increases independently of the duration and severity of the disease, the expression of IL-4 has been detected in many tissues, in brain tissue and cerebral nuclei (in the lateral ventricle) in mice affected by Angiostrongylus (a parasitic infection) [51]. IL-4 expression was detected in the wounds on days 1 to 4 after wounding and then decreased progressively and disappeared on day 21 [52]. Abbas (2017) [54] showed that in cancer cachectic patients, IL-6 produces in large quantities which may be this trigger the different cells to release more cytokines.

Others have shown that expressing IL-4 in tissue improves the immune response against human ovarian melanoma, breast carcinoma [55], and thyroid cancer [20].

Conclusions

These findings indicate that serum levels of IL-4 may help diagnose thyroid cancer and identify patients with the active disease who deserve closer medical attention. Although thyroid cancer does not produce IL-4, it can induce other cells to produce IL-4. The tissue expression of interleukin did not correlate with serum interleukin levels. Furthermore, secretion of IL-4 was systematic and not localized in thyroid cancer tissues. Obesity was not associated with a prevalence of thyroid cancer.

Conflict of interest: none declared

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