RESEARCH ARTICLE

IL-1β a potential factor for discriminating between thyroid carcinoma and atrophic thyroiditis

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ABSTRACT. Interactions between cytokines and others soluble factors (hormones, antibodies. . .) can play an important role in the development of thyroid pathogenesis.The purpose of the present study was to examine the possible correlation between serum cytokine concentrations, thyroid hormones (FT4 and TSH) and auto-antibodies (Tg and TPO), and their usefulness in discriminating between different thyroid conditions. In this study, we investigated serum from 115 patients affected with a variety of thyroid conditions (44 Graves' disease, 17 Hashimoto's thyroiditis, 11 atrophic thyroiditis, 28 thyroid nodular goitre and 15 papillary thyroid cancer), and 30 controls. Levels of 17 cytokines in serum samples were measured simultaneously using a multiplexed human cytokine assay. Thyroid hormones and auto-antibodies were measured using ELISA. Our study showed that IL-1β serum concentrations allow the discrimination between atrophic thyroiditis and papillary thyroid cancer groups ($p = 0.027$).

Key words: IL-1β, cytokines, Bioplex, thyroid

The thyroid gland is susceptible to the development of several diseases including: (i) autoimmune conditions (AITDs) such as Graves' disease (GD), atrophic thyroiditis (AT) and Hashimoto's thyroiditis (HT) and (ii) non-autoimmune diseases such as thyroid nodular goitre (TNG) and thyroid cancer: anaplastic carcinoma (AC), follicular (FC), medullar (MC) and papillary thyroid cancer (PTC). The latter is the most common form of thyroid cancer and was histological classified as mentioned earlier [1]. The thyroid gland is important to the human body because of its ability to produce, in addition to hormones, a variety of immunologically active factors such as cytokines, growth factors, adhesion molecules and inflammatory mediators (nitric oxide and prostaglandins). These molecules have pleiotropic effects, playing critical roles in activation, growth and differentiation of several target cells, and influence susceptibility to many thyroid diseases. Indeed, thyroid cells are now known to produce many cytokines including IL-1, IL-6, IL-8, IL-12, IL-13, and IL-15 [2] and are targets for many other cytokines. The latter upregulate the inflammatory reaction through stimulation of T and B lymphocytes, resulting in antibody production and tissue injury, and play a crucial role in thyroid disease [3, 4]. The expression of intercellular adhesion molecule-1 (ICAM-1) and lymphocyte function-associated antigen-3 (LFA-3) by thyroid cells is enhanced by IFN-γ, TNF, and IL-1. In different experimental systems, IL-1 has been found to stimulate thyroid cell proliferation [5] and inhibit several steps in the synthesis and release of thyroid hormones [6]. In addition, IL-1 enhances the expression of CMHII molecules [7] and adhesion molecules on thyrocytes. It also stimulates the thyroidal production of other cytokines such as IL-6 and IL-8 [8, 9] and disturbs the thyroid epithelial barrier [10]. In the current study, we investigated cytokine levels in serum of patients affected with different thyroid pathologies on the basis of the genetic implication of certain cytokine genes (IL-1 and TNF) in thyroid pathogenesis in the Tunisian population [11, 12]. Our aim was to determine whether cytokine concentrations in blood serum could be used to discriminate between the different thyroid disease states. Our results showed that IL-1β is a factor that may be used to discriminate between PTC and AT.

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SUBJECTS AND METHODS

Subjects

Serum samples were obtained from 115 patients with different thyroid diseases (17 HT, 11 AT, 44 GD, 15 PTC and 28 TNG). These were investigated and compared to serum samples from 30 controls who had no history of thyroid disease. GD was defined by the presence of hyperthyroidism and a diffuse goitre, supported by the presence of either thyroid anti-peroxidase (TPO) and/or anti-thyroglobulin (Tg) auto-antibodies and positive antithyrotropin receptor (R-TSH). HT was diagnosed by the presence of primary hypothyroidism, goitre and the presence of auto-antibodies to TPO, with or without antibodies to Tg. AT was defined by the absence of goitre and decreased levels of T_4 and enhanced levels of TSH. The diagnosis of TNG and carcinoma was performed by scintigraphy. The latter was verified by surgical intervention and was classified after histological evaluation.

Measurement of serum cytokine concentrations

A Bio-Plex human 17-plex cytokine assay kit (Bio-Rad Laboratories, Hercules, CA, USA) was used to assess for the presence of 17 cytokines: IL-1β, IL-2, IL-4, IL-5, IL-6, IL-7, IL-8, IL-10, IL-12 (p70), IL-13, IL-17, TNFα, granulocyte colony-stimulating factor (G-CSF), granulocyte/macrophage colony-stimulating factor (GM-CSF), IFN-γ, macrophage inflammatory protein (MIP)-1β, and monocyte chemotactic protein (MCP)-1. The assay was performed according to the manufacturers' instructions. In brief, the premixed standards were reconstituted in 0.5 mL of a Bio-Plex human serum standard diluent, generating a stock concentration of 50,000 pg/mL for each cytokine. The standard stock was serially diluted in the Bio-Plex standard serum diluent to generate eight points for the standard curve. The assay was performed in a 96-well filtration plate supplied with the assay kit. Premixed beads (50 μL) coated with target capture antibodies were transferred to each well of the filtration plate and washed twice with Bio-Plex wash buffer. The samples were diluted 1:3 in the Bio-Plex serum sample diluent. Premixed standards or diluted samples (50 μL) were added to each well containing washed beads. The plate was shaken and incubated at room temperature for 30 min at low speed (300 rpm). After incubation and washing, premixed biotin-conjugated detection antibodies were added to each well. Then the plate was incubated for 30 min with shaking at low speed (300 rpm). After incubation and washing, streptavidin-phycoerythrin was added to each well. The incubation was terminated after shaking for 10 min at room temperature. After washing, the beads were resuspended in 125 μL of Bio-Plex assay buffer. Beads were read on the Bio-Plex suspension array system (Bio-Rad), and the data were analyzed using Bio-Plex Manager software version 3.0 with 5PL curve fitting.

Measurement of TSH and FT4

Serum TSH and FT4 levels were measured by an immunoenzymometric assay using TSH Human ELISA Kit (TS045T calbiotech) and FT4 Human ELISA Kit (F4107T calbiotech) respectively. Positive values were considered in the ranges [0.34-5.6] mIU/L and [7.5-21.1] pmol/L for TSH and FT4 concentrations respectively.

Measurement of anti-TPO and anti-Tg auto-antibodies

Serum anti-TPO and anti-Tg auto-antibodies concentrations were measured by immunoenzymometric assay (The Binding Site Group Ltd, Birmingham, UK). TPO and Tg auto-antibodies were considered negative when the concentration was under 40 U/mL and 75 IU/mL respectively and were considered positive in the range [315-585] U/mL and [450-750] IU/mL respectively.

Statistical analysis

Student's *t*-tests as well as the Mann–Whitney non-parametric test were used. The correlation between thyroid hormones or thyroid auto-antibodies levels and cytokines levels was assessed using Pearson's correlation coefficients. Linear discriminant analysis was used to determine which variables could discriminate between the five disease groups. *p*-values less than 0.05 were considered to be statistically significant. All statistical analyses were performed using the SPSS package (13.0).

RESULTS

A total of 115 patients with different thyroid diseases and 30 controls were recruited to measure the serum levels of 17 cytokines simultaneously using a highly sensitive cytokine assay. The mean levels of cytokines in each group of patients and controls are given in *table 1*. The general distribution of cytokines differs between the different thyroid pathologies and controls. However, for some cytokines, there was no difference in serum levels in particular groups. This is the case for MCP-1, where the mean values for all groups, except AT, were similar to controls, and for IL-8, where the TNG and AT group means were equal to controls. On the other hand, highly significant differences were found for four interleukins particularly IL-5, IL-7, IL-13 and G-CSF in all pathologies studied (*table 1*).

In order to study the behavior of several variables simultaneously, we performed analysis of variance using the SPSS package performed on the five groups of thyroid conditions (GD, HT, AT, PTC and TNG). Our results showed that the mean levels of IL-7, MIP-1β, IL-1β and IL-5 were significantly different between these groups ($p = 0.002$; $p = 10^{-4}$; $p = 10^{-5}$ and $p = 7 \times 10^{-8}$, respectively).

In a second step, we looked for correlations between thyroid auto-antibodies (anti-Tg and anti-TPO) or hormones (FT4 and TSH) on one side and cytokines on the other. At the anti-thyroid auto-antibodies level, we found that anti-TPO auto-antibodies correlate only with TNFα ($p = 0.03$; $r = -0.18$) in all thyroid conditions. As regards thyroid hormones, significant correlations found in controls and different thyroid conditions are reported in *table 2*. Only FT4 levels displayed a correlation with some cytokines. The most significant correlation was found with IL-5 in affected individuals ($p = 8 \times 10^{-3}$; $r = -0.023$). IL-5 and TNFα correlate with FT4 in both controls and thyroid disease groups (*table 2*).

Moreover, possible correlations between hormones or thyroid auto-antibodies and cytokines were sought in each thyroid disease. No significant associations were found

Table 1
Comparison of the mean levels of cytokines between 30 controls and 115 patients affected with different thyroid pathologies using the non-parametric Mann-Whitney; (mean ± standard deviation). *p* values were mentioned. Serum cytokine concentrations are in pg/mL.

	GD N = 44		HT N = 17		AT N = 11		PTC N = 15		MNG N = 28		Controls N = 30
	(pg/mL)	*p*	(pg/mL)	*p*	(pg/mL)	*p*	(pg/mL)	*p*	(pg/mL)	*p*	(pg/mL)
IL-1 β	6.02 ± 4.31	NS	4.59 ± 5.85	1.6×10^{-2}	65,09 ± 193.56	NS	0,7 ± 1.82	4×10^{-7}	4.01 ± 4.98	6×10^{-3}	15,16 ± 4.8
IL-5	12.38 ± 14.53	3×10^{-5}	4,3 ± 4.6	2.1×10^{-6}	8,81 ± 7.68	9×10^{-4}	4.06 ± 12.7	2×10^{-6}	2.08 ± 2.85	1.3×10^{-9}	33,7 ± 28.39
IL7	34.37 ± 29.28	3×10^{-5}	18.25 ± 18.13	4.5×10^{-6}	21.22 ± 26.37	10^{-4}	17.81 ± 35.4	5×10^{-5}	15.56 ± 19.45	6×10^{-8}	71,83 ± 39.05
IL-12	21.06 ± 38.52	3×10^{-4}	32.51 ± 86.73	5.2×10^{-5}	23.26 ± 38.18	2.6×10^{-2}	7.81 ± 10.1	4×10^{-5}	29.68 ± 62.1	10^{-4}	41,87 ± 48.59
IL-13	21.04 ± 48.67	2×10^{-5}	1,78 ± 3.3	2.7×10^{-7}	10.61 ± 10.69	8×10^{-4}	3.14 ± 7.88	3.8×10^{-6}	4.06 ± 8.11	3×10^{-8}	38,73 ± 38.67
IL-17	4,32 ± 15.8	2×10^{-3}	0.43 ± 1.78	6×10^{-3}	4,94 ± 11.57	NS	3.92 ± 18.4	NS	5.22 ± 21.37	3×10^{-3}	19,03 ± 31.3
G-CSF	59.46 ± 71.57	4×10^{-6}	11.53 ± 21.86	1.8×10^{-7}	34.64 ± 39.4	9×10^{-6}	30.37 ± 73.66	4×10^{-5}	18.68 ± 52.26	7×10^{-9}	137,95 ± 63.5
MCP-1	181.83 ± 221.06	NS	234,03 ± 267.37	NS	282.64 ± 211.25	6×10^{-5}	70.42 ± 46.43	NS	198.25 ± 214.79	NS	96,05 ± 37.6
MIP1β	989.71 ± 1789.52	NS	1375,04 ± 1279	1.2×10^{-7}	1621.5 ± 1243.3	4×10^{-4}	363.35 ± 310	1.8×10^{-5}	1900 ± 2323.42	1.7×10^{-10}	125 ± 53.4
IL2	43.26 ± 51.67	4×10^{-3}	20.75 ± 29.29	10^{-4}	49.48 ± 47	NS	24.98 ± 46.62	8×10^{-4}	122.11 ± 561.38	2×10^{-5}	78,64 ± 54.15
IL4	11.9 ± 55.25	10^{-3}	5,71 ± 23.17	3.7×10^{-6}	1,33 ± 2.16	6×10^{-4}	4.5 ± 14.82	8×10^{-4}	20.76 ± 76.28	3×10^{-8}	4,84 ± 3.47
IL6	16.65 ± 34.92	10^{-4}	0.26 ± 0.75	1.1×10^{-7}	14.43 ± 31.4	3×10^{-3}	10.06 ± 23.07	2×10^{-4}	137.44 ± 284.53	5×10^{-2}	32.58 ± 26.64
IL8	12.86 ± 16.22	6×10^{-3}	14,72 ± 25.1	1.3×10^{-2}	23.95 ± 43.4	NS	10 ± 19.3	10^{-3}	56.48 ± 130.9	NS	19.54 ± 15.14
IL10	18.35 ± 44.61	1.2×10^{-5}	4,13 ± 10.22	4.2×10^{-6}	9.5 ± 11.55	2×10^{-3}	5.87 ± 10.77	3×10^{-5}	13.96 ± 35.45	2×10^{-5}	31.8 ± 28.35
GM-CSF	131.7 ± 173.07	3×10^{-5}	18.7 ± 46.95	1.2×10^{-7}	172.65 ± 187.4	3.6×10^{-2}	99.44 ± 165.67	10^{-4}	118.91 ± 479.9	7×10^{-8}	336,97 ± 232.57
IFN-γ	148.9 ± 170	10^{-4}	51.98 ± 26.7	5×10^{-6}	183.52 ± 149.75	3.7×10^{-2}	122.19 ± 154.8	2×10^{-3}	94.27 ± 110.2	8×10^{-6}	345,13 ± 233.77
TNF-α	12.66 ± 20.35	2×10^{-5}	1,62 ± 4.66	6.7×10^{-6}	22.5 ± 20.3	3.6×10^{-2}	7.1 ± 13.17	10^{-4}	8.25 ± 15.27	2×10^{-5}	61.38 ± 60.75

NS: non-significant.

between thyroid hormone levels, thyroid auto-antibodies versus cytokines for both GD and HT groups. As regards the AT, PTC and TNG groups, the positive correlations are shown in *table 3*.

We used linear discriminant analysis in order to search for a linear combination of features which characterizes or separates two or more classes of objects. We performed this analysis at two levels: i) between AITD entities (GD, TH and AT) and ii) between AITD, cancer and TNG groups. This analysis was did not reveal any discrimination between the AITD entities. However, IL-1β clearly discriminates the controls from the disease groups ($p = 0.022$), and particularly the AT group from controls ($p = 0.013$). IL-1β also allows discrimination between individuals with AT and those with PTC ($p = 0.027$), which have the highest and the lowest average values respectively.

Table 2
Significant correlations between FT4 and cytokines in all thyroid conditions (line 1) and controls (line 2). *P* values and Pearson's correlation coefficients are included.

	MIP1β	IL-13	IL-7	IL-5	TNF-α	GM-CSF	IL-10	IL-1β	IFN-γ	IL-2	IL-6
FT4 (Thyroid affections)	$p = 3.9 \times 10^{-2}$ $r = 0.185$	$p = 2 \times 10^{-2}$ $r = -0.2$	$p = 2.7 \times 10^{-2}$ $r = -0.2$	$p = 8 \times 10^{-3}$ $r = -0.023$	$p = 1.7 \times 10^{-2}$ $r = -0.213$	$p = 10^{-2}$ $r = -0.23$	$p = 3.2 \times 10^{-2}$ $r = -0.193$	NS	NS	$p = 2.710^{-2}$ $r = -0.198$	NS
FT4 (controls)	NS	NS	NS	$p = 2.3 \times 10^{-2}$ $r = -0.414$	$p = 4.8 \times 10^{-2}$ $r = -0.364$	NS	NS	$p = 1.9 \times 10^{-2}$ $r = -0.426$	$p = 3.5 \times 10^{-2}$ $r = -0.386$	NS	$p = 2.8 \times 10^{-2}$ $r = -0.402$

NS: non-significant.

DISCUSSION

Levels of serum cytokines are important markers for a broad range of human health conditions, ranging from infectious disease, autoimmune diseases to cancer. In this work, using a bioplex cytokine assay kit, we simultaneously investigated 17 cytokines in the serum of patients affected by different thyroid diseases. This approach was used in a previous study using a multiplex serum analysis of thyroid diseases that showed that some cytokines (IL-8, IL-12, HGF and MIG) could be used to discriminate between benign and malignant thyroid cancer via a multivariate analysis [13].

We found that IL-1β levels were underexpressed in the PTC group compared to other thyroid diseases and compared to controls ($p = 4 \times 10^{-7}$) (*table 1*). This may be due to repression of IL-1B gene expression at the transcriptional level or to post-transcriptional modifications. We found that IL-1β is a factor discriminating between thyroid conditions by discriminating controls from the disease groups ($p = 0.022$), and secondly between PTC and AT groups ($p = 0.027$). No discrimination value was found either within the AITD group or in others thyroid conditions. In fact, it is well known that IL-1 influences the function of thyroid cells by downregulating the expression of Tg [14] and TPO [15], inhibiting iodide organification [16] and the Na^+/I^- symporter NIS [17], and reducing the delivery of thyroid hormone to the circulation [6]. Furthermore, it was demonstrated that concentrations of IL-1β modify thyroid epithelial tightness of human thyrocytes by altering the expression, localization and organization of junction proteins, confirming the important role played by IL-1β in thyroid pathogenesis (unpublished results).

IFNγ, a prototypic proinflammatory cytokine produced by several different cell types was, in our study, significantly decreased in HT patients compared to other groups and especially *versus* controls ($p = 5 \times 10^{-6}$). These findings confirm results mentioned by Shi *et al.* [18] that plasma IFN-γ concentration and IFN-γ mRNA in peripheral blood mononuclear cells were lower in HT patients than in controls ($p<0.01$). In the present study, the highest serum IFN-γ levels were found in AT patients and the highest serum IL-6 levels were found in TNG patients compared to others pathologies. These findings are concordant with those reported by Zorin NA *et al.* [19] who suggested that an elevated level of IFN-γ in AT contributes to blockade of the endocytosis of peptide hormones and cytokines transported by macroglobulins. Our results are in disagreement in part with those of Phenekos C *et al.* [20], who found that patients with HT had higher INF-γ levels compared to patients with TNG, GD and controls. In our study, patients with GD had higher serum levels of IL-4 in comparison with patients with HT. These results were similar to those reported by Phenekos C *et al.* [20].

The analysis of variance showed that MCP-1 and others cytokines were significantly different in all thyroid diseases. In fact, Kemp *et al.* [21] showed that MCP-1 and some other chemokines were expressed in all Hashimoto's and most Graves' disease thyroid specimens, but very low expression was detected in the non-autoimmune goitre samples.

Concerning auto-antibody correlations with cytokines, our study showed that anti-TPO auto-antibodies correlate with some humoral cytokines: IL-13, IL-6 and IL-4 in the TNG

Table 3
Significant correlations ($p \leq 10^{-3}$) between thyroid hormones and auto-antibodies with cytokines in AT, PTC and TNG groups. *P* values and Pearson's correlation coefficients are included.

	AT	PTC	MNG
FT4	–	IL17 ($p = 3 \times 10^{-3}$; $r = 0.8$) IL12 ($p = 6 \times 10^{-3}$; $r = 0.77$) IL7 ($p = 9 \times 10^{-3}$; $r = 0.74$) IL5 ($p = 7 \times 10^{-3}$; r = 0.75) GM-CSF ($p = 3 \times 10^{-3}$; $r = 0.8$) IL2 ($p = 7 \times 10^{-3}$; $r = 0.75$)	–
TSH	–	—	IL7 ($p = 3 \times 10^{-3}$; $r = 0.58$)
Anti-TPO	IL2 ($p = 7 \times 10^{-3}$; r = 0.75)	–	IL13 ($p = 7 \times 10^{-5}$; $r = 0.72$) IL6 ($p = 5 \times 10^{-3}$; $r = 0.55$) IL4 ($p = 1.4 \times 10^{-10}$; $r = 0.92$)

group. However, in a previous study, no correlation was observed between serum levels of thyroid auto-antibodies and serum levels of cytokines in TNG [22]. In the same study, TNG patients showed increased concentrations of cytokines IL-6, IL-8 and IL-2, which is not the case in our study.

As regards correlations between thyroid hormones and cytokines levels, TSH correlates with IL-7 in the TNG group. In our study, no correlation was observed between FT4 and cytokines in GD patients as suggested in a previous study [22].

By using a highly sensitive Bioplex assay technique, we observed abnormalities in a broad range of cytokines, probably reflecting the complexity of the underlying disease processes present in the different thyroid conditions. Moreover, studies on the changes in serum cytokine levels in thyroid diseases often provide controversial results but they remain essential for understanding the implication of cytokines in thyroid pathogenesis. In addition, serum cytokine levels may not reflect the intrathyroidal levels of some cytokines which may be low in the periphery, despite high tissue concentrations. For this reason investigation of cytokine mRNA expression (namely IL-1β) in thyroid tissues is necessary. Indeed, if confirmed, the role played by IL-1β in discriminating between different thyroid conditions would be an important finding, allowing improvements in both early diagnosis and treatment choice for thyroid disease.

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