Levels of cytokines and thyroid autoantibodies in Omani patients with Graves' disease

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ABSTRACT

An altered balance of pro- and anti-inflammatory cytokines is thought to play an important role in the pathogenesis of autoimmune thyroiditis. The aim of the present study is to assess the cytokine and autoantibody profiles in Omani patients with Graves' disease (GD). Cytokines and autoantibodies including interleukin (IL)-2, IL-4, tumour necrosis factor (TNF)α, interferon (IFN)γ, thyroid stimulating hormone receptor antibody (TRA) and thyroid peroxidase antibody (TPO) are measured in GD patients (n=59) before treatment (n=23) and after treatment (n=36) with ^{131}I-labelled iodine, and compared with normal controls (n=20). Patients with GD showed comparable serum levels of IL-2 but significantly higher levels of IL-4, TNFα, IFNγ, TRA and TPO, compared with the normal controls. There was also a significant increase in serum levels of IL-4 and TNFα, and a decrease in TRA in the treated group, compared to the untreated group. IL-4, TNFα, IFNγ, TRA and TPO showed a high prevalence in Omani patients with GD. Thus, cytokines and autoantibodies may prove useful in the diagnosis of GD and in assessing prognosis.

KEY WORDS: Autoantibodies.
Cytokines.
Graves disease.
Thyroid gland.

Introduction

Graves' disease (GD) is an organ-specific autoimmune disorder characterised by the presence of autoantibodies against thyroid-specific proteins, including the thyroid stimulating hormone (TSH) receptor, thyroglobulin and thyroid peroxidase (TPO).[1–4]

The TSH receptor plays an important role in the function and growth of thyroid cells.[5] The pathogenesis of Graves' hyperthyroidism is characterised by humoral autoimmune responses in which hyperthyroidism is induced by a thyroid stimulating hormone antibody against the TSH receptor (TRA).[6] This is a well-known marker of thyroid gland autoimmunity and may have some predictive value in the recurrence of GD after treatment with antithyroid drugs.[7]

A change in cytokine profile in GD has been proposed as a contributing factor in the induction and maintenance of the autoimmune process.[1,8] Furthermore, serum cytokine levels appear to correlate with endocrine status, indicating that they could function as markers of thyroid activation in GD.[9]

T cells can be divided according to their cytokine profile into two major subsets of T helper (Th) cells. Th1 cells produce cytokines that support inflammation and cell-mediated immune responses associated with the pathogenesis of autoimmune disease (autoimmune thyroiditis), and these include interferon (IFN)γ and interleukin (IL)-2, while Th2 cells release cytokines that promote antibody-mediated immune responses and inhibit Th1 responses (e.g., IL-4).[2,3]

Consequently, measurement of cytokines may be of value in the differential diagnosis of hyperthyroidism and in the follow-up of GD, both during and after treatment with antithyroid drugs, ^{131}I-labelled iodine or surgery. The present study aims to explore the correlation between the use of antithyroid medication and the occurrence of thyroid-specific autoantibodies and cytokines.

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Materials and methods

This study was approved by the Sultan Qaboos University Medical Research and Ethics Committee (Project No. MREC 155).

Subjects

A total of 59 adult Omani subjects (43 females, 16 males; age range: 16–51 years, average 29.7 years) with a clinical diagnosis of GD were included in the study. On the basis of treatment with ^{131}I-labelled iodine, which was the only type of treatment available, patients were allocated to the treated group (n=38) or the untreated group (n=21, newly diagnosed). The control group comprised 20 normal, healthy Omani blood donors matched for age and gender.

Measurement of cytokines

All serum samples were stored frozen at –70°C and then thawed once at the time of assay. IL-2, IL-4 and IFNγ (Quantikine enzyme-linked immunosorbent assay [ELISA], R&D Systems, Minneapolis) and tumour necrosis factor-α (TNFα) (TiterZyme ELISA, DRG International, Mountainside, NJ) were measured following the

Table 1. Serum IL-2, IL-4, TNFα and IFNγ in the control (C), untreated (U) and treated (T) patient (P) groups.

	Controls (n=20)	Untreated (n=21)	Treated (n=36)	P value (U vs. T)
IL-2 (pg/mL)	64.1±9.9	66.9±16.2	67.7±19.7	>0.05
P value (C vs. P)		>0.05	>0.05	
IL-4 (pg/mL)	52.9±14.4	75.9±21.7	89.3±34.9	>0.05
P value (C vs. P)		<0.05	<0.05	
TNFα (pg/mL)	72.5±12.4	80.5±24.1	94.4±25.9	<0.05
P value (C vs. P)		>0.05	<0.05	
IFNγ (pg/mL)	74.4±27.5	89.7±26.1	101.5±29.8	>0.05
P value (C vs. P)		>0.05	<0.05	

manufacturers' instructions. Assay sensitivities were 7 pg/mL for IL-2, 10 pg/mL for IL-4 and TNFα, and 8 pg/mL for IFNγ. Samples were dispensed in 96-well microtitre ELISA plates and incubated at room temperature for 2 h. The plates were then rinsed (x4) with wash buffer and incubated for 2 h with anticytokine horseradish peroxidase conjugate against each of the cytokines tested. The bound enzyme was detected by incubation with tetramethylbenzidine and hydrogen peroxide as a substrate, and then quantified using a microplate reader. Sample variation was estimated at approximately 5%.

Measurement of TRA

Circulating TRA was measured with a commercial ELISA kit (Medizym TRA, Medipan Diagnostica, Germany), following the manufacturer's instructions.

In summary, the method comprised two incubation steps. During the first incubation, TRA was bound to the immobilised receptor on the solid phase of the microtitre plate. In the second incubation, the TSH complex bound to the free epitopes of the receptor.

Bound receptor-TSH complexes react specifically with horseradish peroxidase conjugate, which converts the colourless tetramethylbenzidine to a blue product. This enzyme reaction was stopped by adding H_2SO_4, which turned the solution from blue to yellow. Absorbance (A) was measured at 450 nm, and values >2 units/L were considered positive.

Measurement of TPO

An ELISA assay (Medizym anti-TPO, Medipan Diagnostica, Germany) was used for the quantitative measurement of human TPO antibodies in serum. All procedures were performed according to the manufacturer's instructions. Values for TPO >50 IU/mL were considered positive.

Statistical analysis

Statistical significance was calculated using ANOVA for comparing means and using Pearson's for correlation. Data were presented as mean ± standard deviation (SD), and were analysed using SPSS for Windows. $P<0.05$ was considered significant.

Results

Table 1 shows the results obtained for the cytokines tested in the treated, untreated and control groups, together with P values. No differences based on gender were observed. Table 2 shows the cytokine results with and without TRA and TPO antibodies.

Figure 1 shows the percentage of GD patients who were positive for TRA (73%; 9.6±6.5 U/L) and TPO (69%; 1190.5±1062.6 IU/mL). Values in the normal group for TRA and TPO were 2% (0.514±0.4 U/L) and 6% (20.7±26.2 IU/mL), respectively. Positivity for TRA and TPO antibodies was significantly more frequent ($P<0.05$) in the patients with GD.

When GD patients were subdivided on the basis of treatment with ^{131}I-labelled iodine, a significant difference ($P<0.05$) was observed in TRA and TPO levels between the treated and untreated groups, with lower levels seen in the treated group compared with the untreated group (Table 1).

The results in Figure 2 show that a significant difference ($P<0.05$) was observed in TRA positivity between the treated and untreated groups, with lower levels seen in the treated group (47.2%) than in the untreated group (85.9%). No significant difference was observed between TPO positivity in the treated (69%) and untreated (78.2%) groups.

To ensure that GD patients with TRA and TPO positivity

Table 2. Cytokine concentrations (pg/mL, mean ± SD) with and without TRA (n= 42/17) or TPO (n= 41/18).

	IL-2	IL-4	TNFα	IFNγ
With TRA	65.8±17.1	81.7 ±25.6	87.9 ±24.2	95.6 ±26.7
Without TRA	73.1±20.3	92.5±42.3	93.5±30.7	100.2±34.9
	P>0.05	P>0.05	P>0.05	P>0.05
With TPO	68.5±19.6	84.4±32.3	87.7±25.7	96.0±28.3
Without TPO	64.3±14.4	84.3±28.8	93.8±26.9	100.3±31.1
	P>0.05	P>0.05	P>0.05	P>0.05

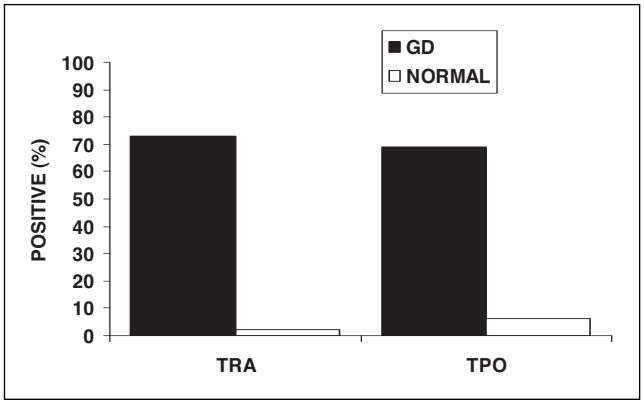

Fig. 1. Patients with Graves' disease screened for serum antibodies to antithyroid-stimulating hormone receptor (TRA) and antithyroid peroxidase (TPO) antibodies. Comparison with normal controls.

did not introduce bias to the calculations, results for GD patients with and without TRA and TPO antibodies were compared, and no significant differences were seen (Table 2).

There was no correlation (r=0.13; $P>0.05$) between TRA and TPO antibodies. Similarly, results obtained failed to show any correlation between TRA or TPO antibodies with any of the cytokines measured ($P>0.05$), either before or after treatment.

Discussion

As has been observed elsewhere,[9,10] serum IL-2 values in GD patients are normal and show no differencebetween treated and untreated groups. Increased serum IL-4 levels have been reported by several investigators,[10–12] all of whom suggested a predominance of Th2 cytokines in GD, indicating a humoral pattern of immune response. The results of the present study support these finding.

In contrast, TNFα is a multifunctional cytokine produced by macrophages and other cells, and is known to play a major role in the immunological cascade that leads to an inflammatory response, with the release of mediators such as IL-2.[13,14] In the present study, serum TNFα levels in GD were increased significantly, mainly in the untreated group, and this supports earlier work.[11,12]

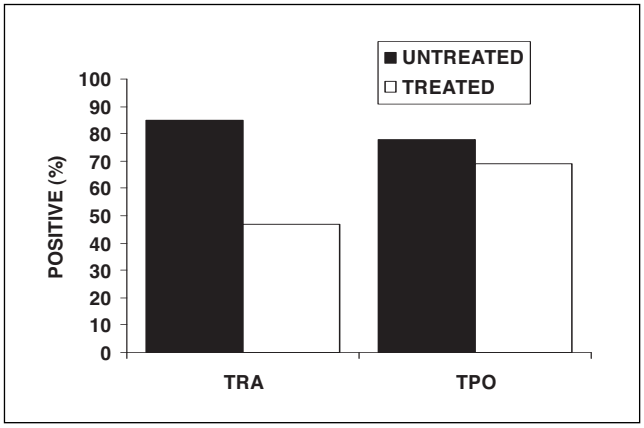

Fig. 2. Untreated and treated patients with Graves' disease who were positive for antithyroid-stimulating hormone receptor (TRA) and antithyroid peroxidase (TPO) antibodies.

Polarisation of intrathyroidal cytokines towards a type 2 profile probably contributes to the production of pathogenic autoantibodies in GD,[15] and type 1 cytokines appear to suppress their production.[16] However, the fact that serum IL-4 and TNFα levels were raised in the GD group, compared with the control group, suggests a mixed Th1 /Th2 response. Moreover, Th1 and Th2 cells show cross-regulation; thus, cytokines produced by one Th subset can suppress the production and/or activity of the other.[17]

Increases in INFγ levels in GD have not been reported previously, nor have differences in levels between treated and untreated GD patients.[10] However, results from the current study show higher values in the GD groups compared with the control group, with significantly higher levels in the treated GD group. This may be due to differences in the number of subjects studied or to the different methods of measurement employed,[18] as well as to differences in genetic and environmental factors.

Treatment with radioactive iodine alters the production of thyroid hormones and autoantibodies in GD. Earlier investigations suggest that TRA levels fall during antithyroid drug treatment, and this may have predictive and prognostic value in the recurrence of GD after treatment.[19,20] The present study supports these findings, and the post-treatment decline in TRA antibody level suggests that the thyrotoxicosis has an autoimmune aetiology.

No changes in TPO level in the treated GD group were seen, and this result is consistent with that from a recent similar study.[20] However, an earlier report[21] described an increase in serum TPO levels after treatment with ^{131}I-labelled iodine, while another study[22] reported a decrease. Such inconsistency may be due to differences in immunological response as disease activity declines, or may be a direct effect of the drug.

The absence of a positive correlation between TRA and TPO in GD patients did agree with results from a previous study.[22] This may have been due to differences in GD pathogenesis or to differences in the prevalence of these autoantibodies among treated and untreated groups. It may also have been due to differences in the number of subjects studied, different assays used or to genetic and environmental factors.

It is possible that interaction between genetic, environmental and endogenous factors initiates autoimmune and inflammatory responses in patients with autoimmune thyroid disease. However, whether or not serum cytokine concentration in GD is influenced by thyroid function or by autoimmune reactions remains unclear. In addition, other factors such as antithyroid therapy and age may have an effect on circulating cytokines. Clearly, further studies are needed in this area. □

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