Increased soluble Fas Serum Level associates with thyroid auto-antibody positivity in untreated Graves' hperthyroidism: A case-control study

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Abstract

Serum soluble Fas (sFas) increases in Graves' disease (GD) and it may play a role in the pathogenesis of GD through interfering with the Fas-FasL interaction.

To determine the association of serum sFas level with thyroid autoantibodies we evaluated the serum level of sFas and thyroid autoantibodies in 31 untreated GD patients and 37 re-lated healthy controls.

Serum sFas level was significantly higher in patients ($P<0.005$) compared to controls. Odds ratios of thyroid autoantibodies serum positivity for serum sFas quartiles were 1.99 (95%CI: 1.2-3.3) for TSH receptor antibodies (TRAb), 1.68 (95%CI: 1.04-2.71) for thyroid peroxidase antibodies (TPOAb), 1.13 (95%CI: 0.7-1.83) for anti thyroid microsomal anti-bodies and 1.17 (95%CI: 0.73-1.88) for anti thyroglobulin antibodies. However, Odds ratios of quartiles of serum titer of thyroid autoantibodies for serum sFas were not significant. There was a moderate but significant direct correlation between serum levels of sFas and free T4 and free T3 ($\rho=0.392$ and $\rho=0.360$ respectively; $P<0.05$) in GD patients.

Our data showed a significant association between thyroid autoantibodies (TRAb and TPOAb) serum positivity and sFas level. It is not clear if sFas elevation is a result of auto-immune process or has a pathogenic significance.

Introduction

The Fas receptor and its natural ligand (FasL, CD95L) are transmembrane proteins that belong to the tumor necrosis factor family of receptors and ligands [1]. They play a major role in maintaining homeostasis in the immune sys-tem [2].The Fas molecule is expressed by various tissues, including the thyroid gland. Activation of Fas antigen on thyrocytes by binding of the FasL, which is present on lymphocytes, is a potential mechanism by which thyroid cells undergo apoptosis [3].

Alternative splicing, results in a soluble form of the Fas molecule (sFas) that lacks the transmembrane region [6]. The killing mechanism of Fas functions via the membrane bound Fas molecule, whereas sFas protects against apop-tosis [7]. sFas is found in low concentrations in the sera of healthy subjects and at elevated concentrations in the sera of patients with autoimmune diseases, malignancy, and inflammatory disorders [8-10]. Therefore elevated serum levels of sFas may have correlation with the activity of these diseases [9,11]. Graves' disease (GD) is an autoimmune thyroid disorder. It is characterized by the hyperplasia of thyrocytes resulting from stimulation by anti-TSH receptor autoantibodies (TRAb) [12].Although thyrocytes from GD patients express both Fas and FasL in vivo, apoptosis is only occasionally found in the GD thyroids [13]. Some studies have addressed the role of sFas, which suppresses Fas-mediated apoptosis in the pathogenesis of GD. They have shown that serum sFas increases in GD and provide evidences for local production of sFas by thyrocytes and its regulation by cytokines [10]. It has been suggested that sFas may also play a role in the pathogenesis of GD through interfering with the Fas-FasL interaction [10,13].

The precise association of serum sFas level with thyroid autoantibodies is the aim of the present study.

Subjects and Methods

Subjects

The subjects of our study were 31 untreated GD patients (mean age, 38.4 ± 15.7 yr) and 37 normal age and sex matched volunteers (34.4 ± 12.6 yr). GD was diagnosed by suppressed levels of TSH and elevated levels of free T3 (fT3) and free T4 (fT4) and positive TRAb. Normal volunteers had no symptoms or history of autoimmune diseases. The study was approved by the local ethic committee. Informed consent was obtained from all sub-jects after the nature of the study had been explained. The followed procedures were in accordance with the Helsinki Declaration.

Analytical determinations

Serum thyroid peroxidase antibodies (TPO), TSH, thy-roglobulin (TG) and anti TG antibodies (TGAb) levels were measured by immunoradiometric assays (RADIM SpA, Rome, Italy). Serum fT3 and fT4 were evaluated using commercially available radioimmunoassay kits (Di-aSorin, Saluggia, Italy). Quantitative determination of TRAb was carried on using another enzymatic immuno-assay (DLD, Hamburg, Germany). Serum sFas level was evaluated through a commercially available ELISA kit (Bender MedSystems, Vienna, Austria). Reference values were 0.2-4.9 μIU/mL for TSH, less than 40 ng/mL for TG, 3.4-8.5 pmol/L for fT3 and 10-30 pmol/L for fT4. The minimum concerned titre for positivity was 50 U/mL for TPO, 70 IU/mL for TGAb and 1.5 U/L for TRAb as recommended by the manufacturers. Serum samples from cases and controls were always analyzed in the same run. The within-assay coefficients of variation was 6.6% for TSH, 7.9% for TPOAb, 6.2% for T4, 8.4% for TGAb, 4.6% for sFas, 8% for TRAb, 8.1% for fT3, 7% for fT4, 5.2% for TG and 4% for T4.

Thyroid sonography

Thyroid sonography was carried on by ultrasound (ESAOTE-BIOMEDICA-AU5) on all patients using a linear 10MHZ probe on supine position. Both lobes of thyroid gland and isthmic portion were scanned. The size and volume of both lobes were calculated. Nodules were evaluated for their size, volume and echo texture (solid-cystic-mixed echo density and calcification). In multi-nodular cases, the volume of the dominant nodule was calculated. The cervical spaces were also surveyed to rule out lymphadenopathy.

Statistical analysis

Adjustment to normal distribution was tested by the Kolmogorov-Smirnov test. For quantitative variables, results are expressed as mean ± SD for normally distributed data, and as median (interquartile range) for nonparametric data. In order to reduce departures from the normal distribution, sFas levels were natural log-transformed for parametric analysis. For comparisons of means between two independent groups of subjects, the Student t -test was used for normally distributed data and the Mann–Whitney U test for nonparametric data. Chi-square test was used for comparing proportions between two groups.

Pearson's correlation was used to assess the strength of association between values with normal distribution. Values not normally distributed were calculated using Spearman's rank correlation test. Differences were con-sidered significant when $P < 0{\cdot}05$.

To compute odds ratios, continuous data were categorized into quartiles using the frequency distribution of cases and controls combined. The logistic regression model was used to calculate odds ratios relative to the lowest quar-tile. Statistical analyses were performed using SPSS 11.5 and Stata/SE 8.0 (Stata Corporation, TX).

Results

The characteristics of patients and related healthy controls are shown in Table 1. No significant differences in age and sex between patients and controls were found. Serum sFas level was significantly higher in patients (P<0.005) compared to controls (Fig 1). Odds ratio of GD for serum sFas quartiles was 2.02 (95%CI: 1.24-3.29) however adjustment for BMI as confounder modified it to 1.98 (95%CI: 1.21-3.24). Odds ratios of thyroid autoantibodies serum positivity for serum sFas quartiles were 1.99 (95%CI: 1.2-3.3) for TRAb, 1.68 (95%CI: 1.04-2.71) for TPOAb and 1.17 (95%CI: 0.73-1.88) for TGAb. How-ever, Odds ratios of quartiles of serum titer of thyroid autoantibodies for serum sFas were not significant (data not shown).

Table 1: Clinical and biochemical characteristics of the study subjects

	Cases N= 31	**Control N= 37**
Sex (M/F)	11/20	12/25
BMI (Kg/m2)*	23.6±4.3	23.8±3.9
AGE (year)*	38.4±15.7	34.4±12.6
TSH (µIU/ml)†	0.115 (0.024-0.217)	1.41 (0.838-210)
fT3 (pmol/l)*	17.7±7.1	7.3±0.87
fT4 (pmol/l)*	61±17.7	16.4±2.5
TG (mol/l†	35.5 (4.6-112.8)§	6.6 (3.7-10.7)
TPOAb (positive/ negative)	22/9¶	7/30
TGAb (positive/ negative)	15/16‡	7/30
TRAb (U/l)†	8.3 (.9-43-3)¶	0.4 (0.3-0.6)
Thyroid (volume (ml)*	15.9±7.6	ND
Nodule volume (ml)†	1.5±1.8	ND
sFas (pg/ml)†	3003 (2273-4228) ‡	2301 (1550-2640)

*Mean ± SD; † MediMean (Interquartile range); ND Not determinened;
§ P <0.05; ‡ P < 0.005; ¶ P < 0.0001

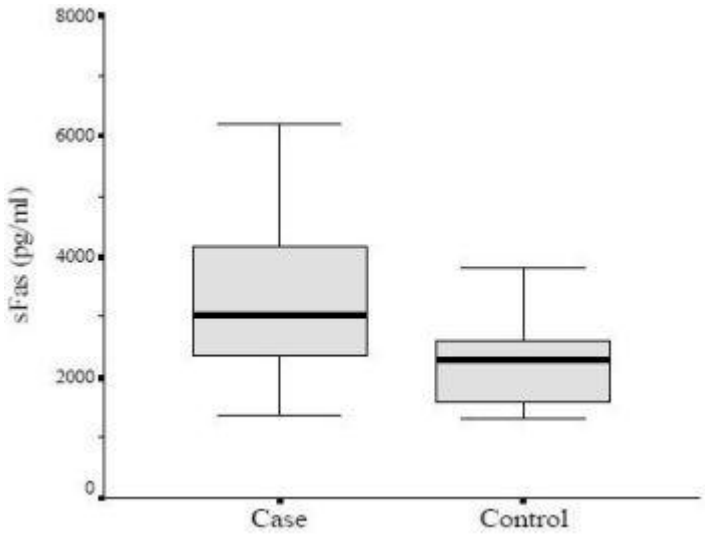

Fig. 1: Serum levels of soluble Fas (s Fas) in cases

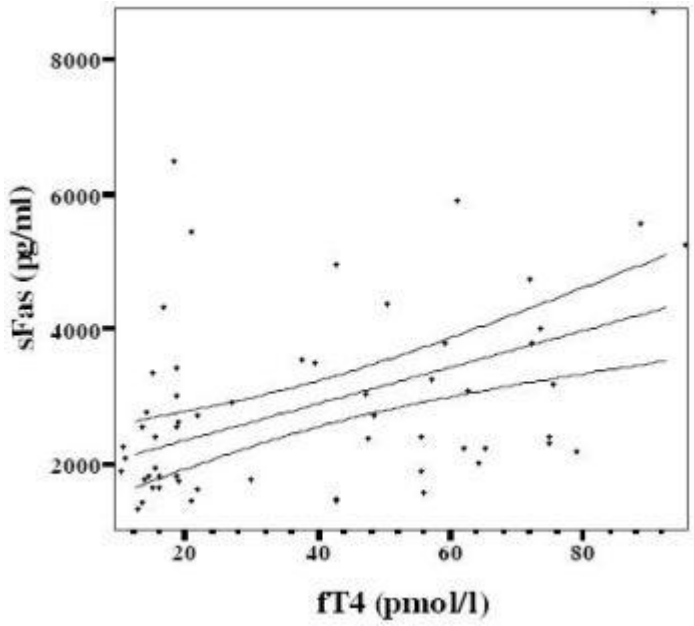

Fig. 2: Correlation between sFas and fT4 in patients and controls ($\rho = 0.525$; $P < 0.001$).

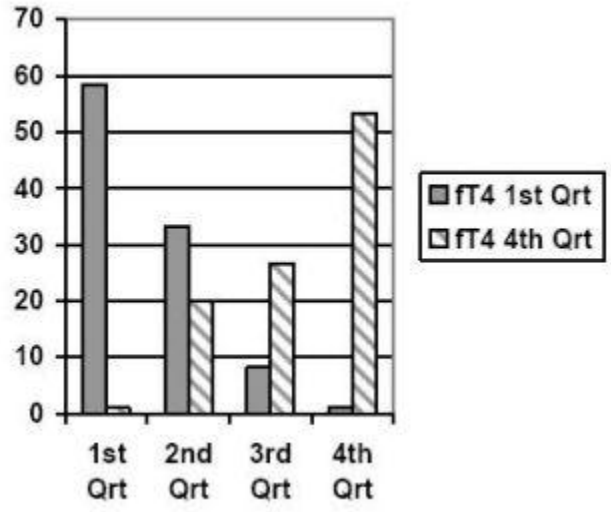

Fig. 3: Trend of serum fT4 quartiles with sFas in patients and controls (P for trend <0.0001)

Discussion

The Fas-FasL system is a primary mediator of apoptosis pathway which has received much attention in terms of a potential role in physiologic homeostasis and immune regulation in various thyroid diseases, including GD [14]. The Fas molecule is expressed by various tissues, includ-ing the thyroid gland [3-5]. Alternative splicing, results in a soluble form of the Fas molecule (sFas) that lacks the transmembrane region [6]. The clinical significance of sFas has not been elucidated completely. Previous studies provided evidences of local production of sFas by thyro-cytes and its regulation by cytokines in GD patients [15]. However Feldkamp et al suggest that the source of sFas in patients with hyperthyroidism is lymphocytes rather than thyrocytes, as patients with only very small thyroid rem-nants and subclinical hyperthyroidism after surgery also had elevated sFas values [16].

The present study confirmed a significant increase in serum sFas level in GD which is in agreement with previous studies [10,15,16].

It is not yet clear if the elevated serum level of sFas results from either the increased production of sFas in thyroid cells and infiltrating lymphocytes, or the increased number of these cells as a result of thyroid hyperplasia. Nevertheless, the observed significant odds ratio of GD for sFas in this study, may indicate the involvement of sFas in the immune process of GD. Liu et al showed an opposite relation between cell surface expression of membrane Fas and production of sFas in activated lym-phocytes [17]. Increased sFas in GD suggests decreased membrane Fas that in turn decreases the potential for apoptosis. On the other hand, sFas is thought to compete for FasL with cell membrane Fas receptor, leading to the inhibition of apoptotic signal transduction [7]. Therefore the increased sFas level is likely to promote thyrocyte proliferation and

production of anti-TSH receptor anti-body by protecting thyroid cells and autoreactive B cells respectively from apoptosis [12]. The observed significant odds ratios of thyroid autoantibodies (TRAb and TPOAb) serum positivity for serum sFas quartiles may reflect the above hypothesis.

The observed significant correlations between sFas and fT4 and fT3 in GD patients and the significant trend be-tween sFas and ft4 in patients and controls together (Fig.3), suggest that sFas may reflect the increased me-tabolism and thyroid hyperplasia or it could be only a sur-rogate of autoimmunity. This is in concordance with other observation indicating that increases in sFas levels are much more a consequence of hyperthyroidism itself than a sequel of autoimmune thyroid diseases [16].

In this study, we demonstrated a significant association between thyroid autoantibodies (TRAb and TPOAb) se-rum positivity and sFas level, which may suggest a role for sFas in the immune process in GD.

Abbreviations

Fas lipid; FasL Fas ligand; sFas soluble Fas; GD Graves' disease; TRAB anti TSH receptor antibodies; fT3 free T3; fT4 free T4; TPOAb, thyroid peroxidase antibodies; TG thyroglobulin; TGAb anti thyroglobulin antibodies.

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