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SFAS AND FASL IN ETIOPATHOGENESIS OF AUTOIMMUNE DISEASES OF THE THYROID GLAND AND NODULAR FORMS OF GOITER

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Abstract

The objective of this research was to check whether the apoptotic inhibitor sFas (soluble fibroblaste-assosiated cell surface) and the apoptotic inducer FasL (fibroblaste-assosiated cell surface ligand) are differentially present in two opposite phenotypes of autoimmune thyroid disorders (AITD) and nodular goiter (NG). However, in contrast to GD (Graves' disease), decreased sFas in HT (Hashimoto's thyroiditis) increased FasL, indicate destruction of thyrocytes. In cases of thyroid NG, sFas may provide a key protective signal that helps the cells to avoid apoptosis in a hostile environment.

Keywords: Autoimmune thyroid disease, Fas ligand, soluble Fas, Graves' disease, Hashimoto's thyroiditis, nodular goiter.

Introduction. The thyroid gland (TG) is the most important organ in the structure of endocrine diseases. In Ukraine these diseases reached 46.67% as of 01.01.2011 [1]. The main reason is, on the one hand environmental degradation in the country, increasing stressful of social origin, on the other hand improved diagnosis of this disease. Regardless the fact that the study of thyroid disease was always given due attention by leading thyroid specialists in the world, this issue is very relevant and requires further study. The autoimmune attack on the thyroid gland results in two opposing clinical syndromes - Hashimoto's thyroiditis and Graves' disease. In HT the lymphocytic infiltration of the thyroid gland causes apoptosis of thyroid cells and hypothyroidism [6, 7, 9, 17, 27]. In contrast, in GD the lymphocytic infiltration of the thyroid leads to activation of thyrotropin receptor (TSHR)–reactive B-cells that secrete TSHR-stimulating antibodies causing hyperthyroidism [13, 22]. The etiology of HT and GD involves common pathways in which thyroid reactive T-cells escape tolerance and infiltrate the thyroid, and unique pathways in which these thyroid-reactive T-cells either cause thyroid cell death (in HT) or stimulation (in GD). Thus, it is not surprising that the genetic susceptibility to HT and GD includes shared genes, as well as unique genes [9, 24, 30]. The processes involved in apoptosis are tightly regulated. Alterations in their functioning may lead to disorders such as autoimmune diseases and cancer) [4]. Apoptosis may play an important role in the homeostasis of thyroid follicular cells as well as destructive mechanisms in thyroid disease. Apoptosis is a mechanism by means of which cytotoxic T-lymphocytes can destroy thyrocytes in thyroiditis, causing hypothyroidism) [18, 25]. In contrast, the suppression of apoptosis may contribute to proliferative diseases of the thyroid gland, such as goiter, cancer, and GD [1, 2, 4, 10, 11]. However, We do not know much about the mechanisms and regulation of apoptotic signaling in thyroid cells. It is important to identify the signaling components of apoptosis in thyroid follicular cells. These signaling components may help in providing insights into potential pathogenic mechanism and lead to development of pharmacological interventions for the treatment of thyroid disease [1, 3, 19, 28]. One of the best characterized death receptors is Fas known as CD95/APO-1/TNFRSF6. It is a 36 KDa cell surface type-1 membrane glycoprotein. Fas ligand (FasL) is a type-II transmembrane protein of the same family, which has a property to bind Fas [5, 14, 20]. Fas have proved to be an important mediator of apoptotic cell death. It is also involved in inflammation. Binding of FasL induces trimerization of Fas in the target cell membrane. Activation of Fas causes the recruitment of Fas associated protein with death domain (FADD) via interactions between the death domain of Fas and FADD. Fas can occur both as a cell surface protein and a soluble protein. Cell surface Fas is anchored by a single membrane-spanning domain and is widely

expressed in normal and malignant cells [1, 2, 8, 21]. Soluble Fas (sFas), alternatively spliced Fas mRNA that results from deletion of exons 3, 4, 6 & 7 and lacks 21 amino acid residues that consist of a transmembrane domain. SFas protects target cells from Fas-induced apoptosis by competitively binding with FasL and altering lymphocyte development and proliferation in response to self-antigens [2, 8, 23, 26]. The Fas pathway has been demonstrated to be the important apoptotic pathway in the thyroid gland. Its role in the pathogenesis of thyroid diseases, however, is controversial and a subject of much debate [26, 29]. Regulation or modulation of this pathway can occur at multiple levels throughout the pathway. This may include changes in the level of the expression of Fas or its ligand [8, 11]; regulation of components of intracellular signaling) [1, 3, 19, 28] and expression of proteins that promote survival, such as members of the Bcl-2 gene family) [15, 16]. This study summarizes the role of Fas-mediated apoptosis in thyroid diseases such as goiter, thyroid cancer, and autoimmune thyroid diseases. Besides, markers of humoral immune response (thyroglobulin and thyroid peroxidase antibodies) were also assessed to compare with sFas and FasL levels.

Materials and Method

A total of 70 patients with thyroid disorder (age range: from 19-to-78 years) were involved in the current study. All the patients were recently diagnosed and untreated for their condition at the time of blood tests. Also, due and required consent was given by each patient to enroll in this study prior to blood collection. The study was approved by Institutional Scientific Review Board & Ethics Committee. The diagnosis of GD and HT was based on commonly accepted clinical and laboratory criteria. In case of NG, blood samples collected prior to surgery and diagnosis was based on histopathological investigation. Of all 70 patients, 47 (67.1%) had AITD, and 23 (32.9%) had NG. For comparison with patients, 20 age matched, healthy individuals were included. Their function test results were normal, thy did not have goiter, and were negative for antithyriod autoantibodies. Venous blood samples were collected in vacutainer tubes between 9:00 to 11:00 am. The samples were allowed to clot for 30 minutes at room temperature. Sera were obtained by centrifugation, were aliquoted, and stored at -20°C till analysis. All the samples were analyzed for thyroid hormone levels and thyroid autoantibodies.

Evaluation of Soluble Fas and Fas Ligand. Circulating sFas and FasL were evaluated by means of sandwich ELISA (Quantikine, R&D systems, USA) kits. Sample activation and dilution were carried out according to the manufacturer's protocol. The unit of measure for the levels of sFas and FasL was ng/mL. The detection range was from 0-2.0

ng/mL and 0-1.0 ng/mL for sFas and FasL, respectively. All enzymatic reaction products were determined photometrically at 450 nm by Plate reader (Thermolabsystems, Finland; Multiskan spectrum).

Estimation of Thyroid Autoantibodies. We used commercially available kits (Immuno-tech, France) to measure serum anti-thyroglobulin (anti-TG), and anti-thyroid peroxidase (anti-TPO) antibodies with the automated gamma counter (Packard, cobra). The detection range was from 0-to-250.0 ng /mL and 0-2100.0 IU /mL for TG and TPO, respectively. We based our measurements on radioimmunoassay and immunoradiomatric assay.

Statistical Analysis. All the statistical analysis was performed with the SPSS 17.0 software and statistical significance was computed by students' i-test and Anova. Receiver's operating characteristic (ROC) curve was also constructed to determine the discriminating efficacy of sFas and FasL. $\boldsymbol{p} < 0.05$ was considered as a statistically significant. The correlation of TPO and TG with sFas and FasL was done by parametric analysis, i.e. Pearson's correlation and non-parametric, i.e. Spearman's correlation test.

Results. A total of 70 samples and 20 controls were analyzed for sFas and FasL levels. Higher average levels of sFas and FasL observed in thyroid diseases were statistically significant when compared to the control ones. The results of circulating serum sFas and FasL levels in the examined thyroid disease patients are demonstrated in Figure 1 as average ± standard error of the average in ng/mL. Levels of sFas were higher in all experimental groups as compared to the control ones. However, the highly significant values were found in GD patients as compared to control ones(0.802 ± 0.059 vs 0.589 ± 0.014, $\boldsymbol{p} = 0.0001$) and compared to HT (0.802 ± 0.059 vs 0.629 ± 0.039, $\boldsymbol{p} = 0.068$).

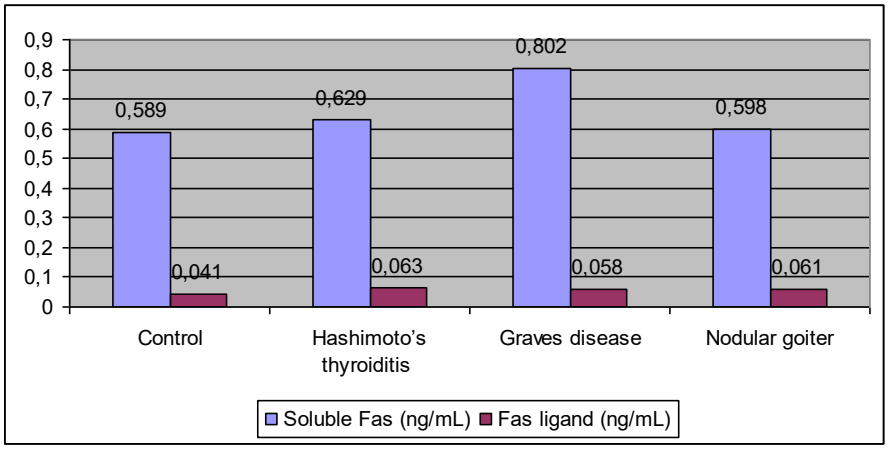


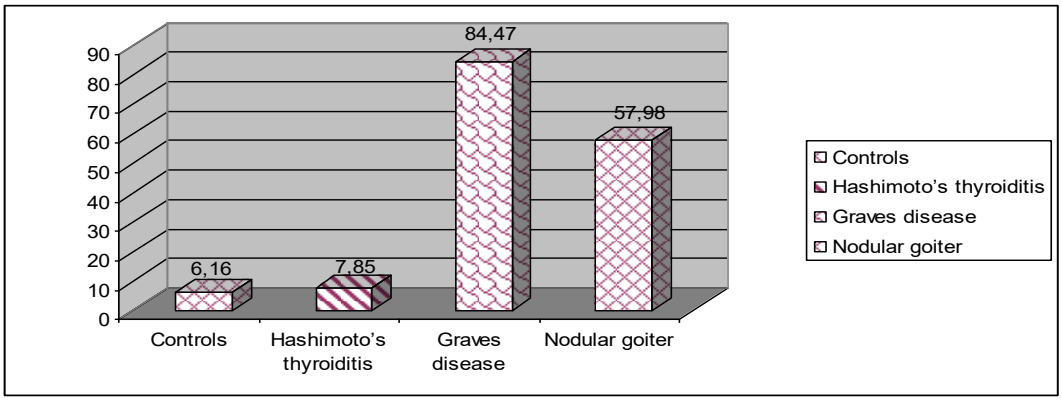
Figure 1. Significance of Soluble Fas and Fas ligand levels in patients with various thyroid diseases as compared to controls.

The difference in FasL levels between GD (0.058 ± 0.003) and HT (0.063 ± 0.007) was not significant.

Significantly higher levels of FasL were observed for NG patients compared to controls (0.061 ± 0.005 vs 0.041 ± 0.006, $p = 0.003$).

Levels of TPO and TG antibodies were also measured and found higher in all groups except TG in the HT group (Figure 2a and 2b). Parametric and nonparametric correlation tests showed significant linear correlations between sFas and TG ($r = 0.319$, $p = 0.05$), TPO ($r = 0.384$, $p = 0.019$). Significant linear correlations were also found between sFas and TPO antibodies ($r = 0.590$, $p = 0.021$) in HT patients and between sFas and TG ($r = 0.543$, $p = 0.011$) in NG patients.

2a



2b

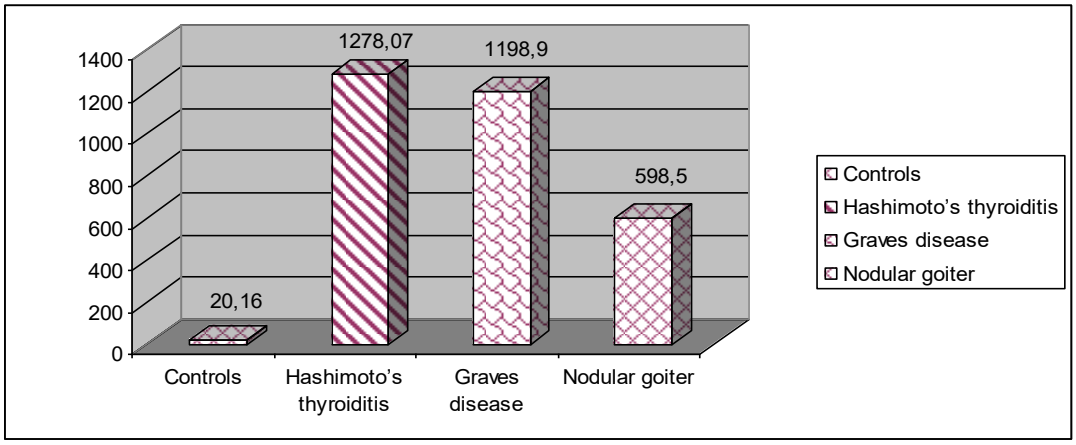


Figure 2. Levels of thyroid peroxidase (fig. 2a) and thyroglobulin (fig. 2b) in patients with various thyroid diseases and controls

There was no correlation between FasL and TG or TPO ROC curve indicates that both sFas and FasL exhibited a good discriminatory efficacy between controls and GD patients (sFas: AUC-0.856; FasL: AUC-0.801) (Figure 3).

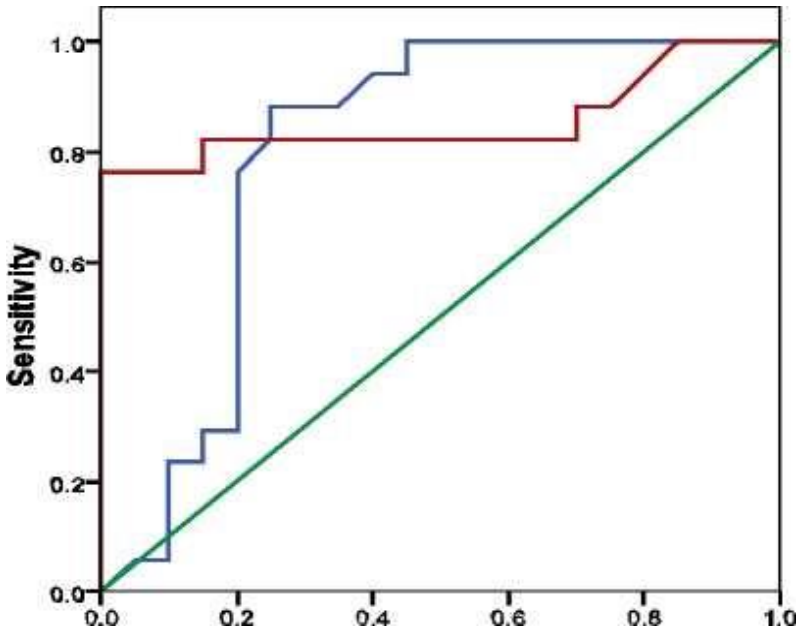


Figure 3. ROC curve for Soluble Fas (red line) and Fas ligand (blue line) between Graves's disease patients with controls

Conclusions. Our study shows that circulating sFas and FasL concentrations are higher in patients with AITD, especially those with GD. This is indicative of enhanced thyroid cell proliferation by protecting against thyroid cell from Fas-mediated apoptosis. However, in contrast to GD, decreased sFas in HT increased FasL, indicate destruction of thyrocytes. In cases of thyroid NG, sFas may provide a key protective signal that helps the cells to avoid apoptosis in a hostile environment.

Prospects of further research: consist in further genetic studies on the role of polymorphism of FAS gene in pathogenesis of autoimmune diseases of the thyroid gland and nodular forms of goiter.

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