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The blood concentration of intercellular adhesion molecule-1 (sICAM-1) and vascular cell adhesion molecule-1 (sVCAM-1) in patients with active thyroid-associated orbitopathy before and after methylprednisolone treatment

Stężenie międzykomórkowej cząstki adhezyjnej-1 (sICAM-1) oraz naczyniowej cząstki adhezyjnej-1 (sVCAM-1) w surowicy krwi chorych z aktywną formą orbitopatii tarczycowej przed oraz po leczeniu metylprednizolonem

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Abstract

Background: The soluble forms of vascular cell adhesion molecule-1 (sVCAM-1) and intercellular adhesion molecule-1 (sICAM-1) have been found to be increased in the blood of patients with Graves' disease. The aim of this study is evaluation of the serum concentrations of soluble forms of adhesion molecules ICAM-1 and VCAM-1 in patients with thyroid-associated orbitopathy (TAO) before and after methylprednisolone treatment.

Material and methods: The study was performed in 40 Graves'disease, hyperthyroid and euthyroid patients with a clinically active form of TAO. Serum concentrations of sVCAM-1 and sICAM-1 in TAO patients were determined by enzyme-linked immunoabsorbent assay (ELISA) before and after intensive pulse methylprednisolone treatment.

Results: We did not find any significant changes in the studied parameters between TAO patients with hyperthyroidism and those with euthyroidism. The serum concentrations of sICAM-1 and sVCAM-1 were significantly increased in patients with TAO before methylprednisolone therapy when compared with the control group. After treatment serum concentrations of sICAM-1 and sVCAM-1 decreased significantly but were still significantly higher than for the control group.

Conclusion: From the results obtained we can conclude that Graves' orbitopathy itself but not thyroid function is probably responsible for the elevated level of the adhesion molecules studied.

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Key words: *adhesion molecules, thyroid-associated orbitopathy*

Streszczenie

Wstęp: Stężenie rozpuszczalnych form cząstek adhezyjnych między innymi międzykomórkowej cząstki adhezyjnej-1 (sICAM-1) oraz naczyniowej cząstki adhezyjnej-1 wzrasta w surowicy krwi chorych na chorobę Gravesa. Celem pracy była ocena stężenia sICAM-1oraz sVCAM-1 u chorych na chorobę Gravesa powikłaną aktywną formą orbitopatii tarczycowej przed oraz po leczeniu immunosupresyjnym.

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Materiał i metody: Badania przeprowadzono w grupie 40 chorych na chorobę Gravesa (w stanie hyper- oraz eutyreozy) powikłaną wystąpieniem aktywnej formy orbiopatii tarczycowej. Stężenie sICAM-1oraz sVCAM-1 w surowicy krwi oznaczano metodą immunoenzymatyczną (ELISA) przed oraz po leczeniu pulsami dożylnymi metylprednisolonu.
Wyniki: Nie wykazano istotnej statystycznie różnicy w stężeniu badanych cząstek adhezyjnych między chorymi na chorobę Gravesa w stanie nadczynności tarczycy oraz w eutyreozie powikłaną aktywną orbitopatią tarczycową. Stężenie sICAM-1oraz sVCAM-1 było znamiennie statystycznie podwyższone u chorych przed leczeniem pulsami metylprednisolonu w porównaniu z grupą kontrolną. Po leczeniu stężenie cząstek adhezyjnych uległo znamiennemu statystycznie obniżeniu.
Wnioski: Na podstawie uzyskanych wyników można wnioskować, że prawdopodobnie orbitopatia tarczycowa, a nie stan metaboliczny tarczycy, odpowiedzialny jest za wzrost stężenia badanych cząstek adhezyjnych.

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Słowa kluczowe: *cząstki adhezyjne, choroba Gravesa, orbitopatia tarczycowa*

Introduction

Thyroid-associated orbitopathy (TAO) is an autoimmune condition characterised by mononuclear cell infiltration of the extraocular muscles (EOM) and/or the orbital fat/connective tissue and proliferation and differentiation of fibroblasts to fibroadipocytes and adipocytes with associated deposition of glycosaminoglycans in the interstitial spaces [1].

Adhesion molecules play an important role in the initiation and maintenance of the inflammatory immune process. Cellular activation and local expression of adhesion molecules lead to leukocyte recruitment, migration to inflammatory sites and targeting in the extravascular space [2]. Cell adhesion molecules mediate rolling and transendothelial migration of circulating leucocytes and may thus direct inflammatory cells into the intima [3]. Soluble forms of the adhesion molecules are detectable in the plasma. The physiological functions of these soluble forms are unclear but their concentration may reflect the expression on the leucocytes and endothelial cells. Soluble forms of various cell adhesion molecules, such as intercellular adhesion molecule-1 (ICAM-1), vascular adhesion molecule-1 (VCAM-1), endothelial leukocyte adhesion molecule-1 (ELAM-1) and E-selectin, may be related to the effects of various diseases [4–6] although the results are inconsistent and often ambiguous.

The aim of this study is to evaluate serum concentrations of soluble forms of adhesion molecules ICAM-1 and VCAM-1 and the possible role of these adhesion molecules as laboratory markers in the assessment of TAO activity.

Material and methods

The study was performed on a group of 40 patients with a first episode of a clinically active form of TAO (clinical activity score > 4 points) and a severity of orbitopathy classified as stages 3c to 5a in the NO SPECS system (0, no signs or symptoms; 1, only signs, no symptoms; 2, soft tissue involvement; 3, proptosis; 4, extraocular muscle involvement; 5, corneal involvement; 6, sight loss due to optic nerve involvement). Of these patients 20 were in hyperthyreosis (group 1) and 20 in euthyreosis (group 2) and all were on thiamazole therapy. The control group consisted of 30 healthy age-matched subjects. In the numerical system given each class of eye change received a score on a scale from 0 to 3 according to the degree of involvement; after the scores were totalled, a Total Eye Score was derived, ranging from 0 to 15 points.

The basal characteristics of the patients with TAO are presented in Table 1. The inclusion criteria for the study were as follows:

— diagnosis of Graves' disease based on clinical and laboratory findings: fT4, fT3, TSH, TSH-receptor antibodies (TRAB), anti-thyroid peroxidase antibodies (TPO-Ab) and thyroid USG scans;
— an ophthalmological examination confirming an active stage of TAO: clinical activity score (CAS) > 4 points [7] and magnetic resonance (MR) scans of the orbit.

Exclusion criteria were any other autoimmune or active infectious diseases. Serum concentrations of sVCAM-1 and sICAM-1 were determined by enzyme-linked immunoabsorbent assay (ELISA, Quantikine, R&D Systems, USA) before and after intensive pulse methylprednisolone treatment (1.0 g per day every other day to the cumulative dose of 9.0 to 12.0 grams during 3–4 week). Intra-assay precision for VCAM-1 was 3.4% and for ICAM-1 4.5%. The differences in distribution of the studied parameters between the TAO patients and the control group were evaluated by the χ^2 test. The statistical analysis was carried out using the "Statistica 5.0 Pl" programme. Data were analysed by the non-parametric Mann-Whitney U-test, assuming the levels $p < 0.05$ as statistically significant.

All the subjects gave their formal consent before participating in the study, and the research followed

Table I

Basal clinical characteristics of the patients and serum concentrations of the soluble intercellular adhesion molecule-1 (sICAM-1) and vascular adhesion molecule-1 (sVCAM-1) in hyperthyroid and euthyroid patients with active thyroid-associated orbitopathy (TAO)

Tabela I

Wyjściowa charakterystyka i stężenia rozpuszczalnej międzykomórkowej cząstki adhezyjnej-1 (sICAM-1) i naczyniowej cząstki adhezyjnej-1 (sVCAM-1) w surowicy krwi u osób z aktywną formą orbitopatii tarczycowej (TAO) w stanie hyper- oraz eutyreozy

	Group 1 hyperthyroid TAO patients (n=20)	Group 2 euthyroid TAO patients (n=20)	p Mann-Whitney U-test
Age (years)	45.11±9.07	53.08±8.82	NS p=0.057
Thyrotropin (TSH) [μIU/ml]	0.2±0.5	3.38±1.8	p<0.05
free T4 [ng/dl]	3.42±2.91	1.26±0.4	p<0.05
CAS (clinical activity score) (points)	5.10±1.85	5.16±0.83	NS p=0.911
Total Eye Score (points)	9.15±2.1	8.34±1.5	NS p=0.24
TRAB [IU/l]	21.7±4.55	18.9 ±2.38	NS p= 0.062
TPO-Ab [IU/l]	257.50±401.91	216.65±288.49	NS p=0.798
sICAM-1 before methylprednisolone therapy [ng/ml]	148.55±7.69	151.65±10.35	NS p=0.441
sICAM-1 after methylprednisolone therapy [ng/ml]	134.69±6.77	136.95±7.00	NS p=0.451
sVCAM-1 before methylprednisolone therapy [ng/ml]	1119.82±112.82	1180.68±142.63	NS p=0.287
sVCAM-1after methylprednisolone therapy [ng/ml]	1020.57±73.92	1076.67±130.11	NS p=0.241

values are means ± standard deviation, TAO - thyroid-associated orbitopathy, TRAB — TSH-receptor antibodies, TPO-Ab — anti-thyroid peroxidase antibodies, sICAM-1 — soluble intercellular adhesion molecule-1, sVCAM-1 — soluble vascular adhesion molecule-1, CAS — Clinical Activity Score of TAO, NS — not significant

the tenets of the Declaration of Helsinki. The project was carried out with the permission of The Bioethical Board of the Medical University of Silesia NN 013–275/01/02.

Results

The results of the study are presented in Tables 1 and 2. We did not find any significant changes in sVCAM-1 and sICAM-1 blood concentration between the TAO hyperthyroid patients (group 1) and the TAO euthyroid patients (group 2). See Table 1.

As there was no statistical difference in the studied parameters between patients from group 1 and those from 2, all the patients were considered as a new study group (group 3: patients with active TAO, n = 40) in the further statistical analysis.

Serum concentrations of sVCAM-1 were significantly increased in patients from group 3 (with TAO) before methylprednisolone therapy when compared with the control group (1153.01 ± 130.67 ng/ml *vs.* 910.69 ± 37.26 ng/ml $p < 0.0005$). After treatment serum concentrations of sVCAM-1 decreased (1051.17 ± 109.67 ng/ml) but were still significantly higher than for the control group ($p < 0.0005$) (Table 2). Serum concentrations of sICAM-1 were also significantly increased in patients with TAO before methylprednisolone therapy when compared with the control group (150.245 ± 9.16 ng/ml *vs.* 128.79 ± 6.68 ng/ml, $p < 0.0005$) and also significantly decreased after methylprednisolone therapy (135.92 ± ± 6.83 ng/ml) (Table 2). Serum concentrations of sVCAM-1 and sICAM-1 in patients with active thyroid-associated ophthalmopathy decreased significantly after methylprednisolone treatment in comparison with concentrations before treatment (Table 2).

Discussion

Proinflammatory cytokines induce the expression of endothelial cell adhesion molecules on the lumenal surface of the vascular endothelium, subsequently increasing leukocyte adhesion, which is the principal mechanism of inflammation [8].

Interactions between activated T lymphocytes and orbital endothelial cells are mediated by integrin-dependent ICAM-1/LFA-1 and VCAM-1/VLA-4 pathways [2]. Positive staining for ICAM-1, lymphocyte function-associated antigen-1 (LFA-1) and very late antigen-4 (VLA-4) were found on infiltrating mononuclear cells. Postcapillary vascular endothelial cells expressed increased ELAM-1, but not VCAM-1. These results suggest that the LFA-1/ICAM-1 and ELAM-1 pathways may be responsible for the migration of mononuclear cells into the thyroid glands of patients with Graves' disease and that the VLA-4/VCAM-1 pathway plays a critical role in the cellular interactions that lead to the formation of B-memory cells and the excess production of antibo-

Table II

Serum concentration of soluble intercellular adhesion molecule-1(sICAM-1) and vascular adhesion molecule-1 (sVCAM-1) in control group and in patients with active thyroid-associated orbitopathy (TAO) before and after methylprednisolone therapy

Tabela II

Stężenia rozpuszczalnej międzykomórkowej cząstki adhezyjnej-1 (sICAM-1) i naczyniowej cząstki adhezyjnej-1 (sVCAM-1) w surowicy krwi u osób z grupy kontrolnej i u chorych z aktywną formą orbitopatii tarczycowej (TAO) przed i po leczeniu metylprednizolonem

	sICAM-1	sVCAM-1
Control (n=30) [ng/ml]	128.79±6.68	910.69±37.26
Group 3 TAO before methylprednisolone treatment (n=40) [ng/ml]	150.245±9.16 p<0.0005*	1153.01±130.67 p<0.0005*
Group 3 TAO after methylprednisolone treatment (n=40) [ng/ml]	135.92±6.83 p<0.05** p<0.0005***	1051.17±109.67 p<0.0005** p<0.0005***

TAO — thyroid-associated orbitopathy, *control *vs*. patients with TAO before methylprednisolone therapy, **control *vs*. patients with TAO after methylprednisolone therapy, ***patients with TAO before methylprednisolone therapy *vs*. patients with TAO after methylprednisolone therapy

dies [9]. Higher soluble ICAM-1 concentrations in patients with Graves' disease with TAO than those with Graves' disease without orbitopathy can reflect the degree of inflammatory activity. Increased soluble ELAM-1 concentrations in patients with TAO only may suggest that soluble ELAM-1 could be a specific marker of endothelium activation in TAO [2]. Pappa et al. found that in early untreated active TAO the interstitial and perimysial connective tissue surrounding the extraocular muscles fibres and numerous mononuclear cells stained strongly for ICAM-1. Vascular endothelial cells stained strongly for VCAM-1 and ICAM-1 [10]. Similar results have been reported by other authors [11]. In late disease the same distribution of immunoreactivity for ICAM-1 and VCAM-1 was observed but with significantly lower staining. The authors concluded that an increased expression of adhesion molecules correlated with early active disease and was reduced in later stages in a way consistent with the results of our study [10].

Wenisch et al. found that serum levels of sICAM-1 and sVCAM-1 were markedly elevated in patients with Graves' diseases before treatment with thiamazole [12]. The serum levels of sELAM-1 and sVCAM-1 decreased significantly after thiamazole therapy and were within the normal range after 4 and 8 weeks of therapy respectively. Serum levels of sICAM-1 were elevated even after 8 weeks of therapy. The authors found that serum levels of sVACM-1 and sICAM-1 correlated with the serum concentrations of anti-thyroid-stimulating hormone (TSH)-receptor antibodies (TSHR-R) and anti-thyroid peroxidase antibodies (TPO-Ab), which is consistent with the results of our study. Similar results have been reported by other authors [13, 14], who found that serum levels of sICAM-1, sVCAM-1 and sP-selectin were markedly elevated in patients with Graves' disease before treatment with methimazole. After 24 months of therapy serum concentrations of sVCAM-1 and sP-selectin were normalised, whereas serum levels of sICAM-1 remained elevated. Serum levels of sICAM-1, sVCAM-1 and sP-selectin in patients with Graves' disease correlated with the serum concentrations of triiodothyronine and thyroxine. Moreover, a positive correlation has been found between serum levels of TPO-Ab, TG-Ab, TRAb and sICAM-1 and sVCAM-1 [13]. In a recent study, Kulig et al. [15] found that in patients both at an active stage of the disease and with fibrotic changes in muscles on MR scans responded well to therapy in parallel with a significant decrease in levels of sICAM-1 and sVCAM-1. Levels of sVCAM-1 increased slightly under prednisone treatment despite an improvement in the clinical picture of TAO and a significant decrease in sICAM-1 levels and in the number of muscles with active inflammatory processes on MR scans. It was concluded that serum levels of ICAM-1 seem to be more sensitive as a marker than MR in assessment of the activity of TAO, but sVCAM-1 does not correspond to the clinical picture of the disease [14]. In contrast to our results, those of Komorowski et al. [6] indicated that thyroid function, but not the presence of TAO itself, is probably responsible for the elevated levels of sICAM and sVCAM. After methyloprednisolone treatment significantly increased levels of sICAM and sVCAM were still found in hyperthyroid patients compared with the control group, but normal serum levels of sVCAM before and after methylprednisolone treatment were found in euthyroid TAO patients [6].

The reduction of ICAM-1 and/or VCAM-1 in relation to methylprednisolone treatment has also been reported in other autoimmune and inflammatory disorders [16]. It is suggested that the ICAM-1 and/or VCAM-1 reduction is due to a peripheral systemic effect on immune-competent cells, and so the observed reduc-

tion might also be a non-specific effect of the methylprednisolone treatment. The effect of methylprednisolone on the blood concentration of adhesion molecules may not be specific for patients with thyroid-associated orbitopathy.

Conclusion

On the basis of our results we conclude that inflammation in the orbit and/or in the thyroid gland itself, but not thyroid function, is probably responsible for the elevated level of the studied adhesion molecules. Evaluation of adhesion molecule concentrations in the blood of patients with TAO, in association with clinical signs (CAS) and orbit MR scans, could be one of the parameters for the evaluation of TAO activity and response to the immunosuppressive treatment, although further studies are still necessary.

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