ORIGINAL ARTICLE

Vascular Cell Adhesion Molecule-1 (VCAM-1) in Graves' Disease: Its Association to Thyroid Status and Thyroid Receptor Stimulating Antibodies

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Soluble vascular cell adhesion molecule-1 (sVCAM-1) and intercellular adhesion molecule-1 (sICAM-1) have been shown to be elevated in patients with Graves' disease and may play significant roles in the pathogenesis of the disease. The objective of this study was to measure the levels of sVCAM-1, sICAM-1, IL-6 and thyroid receptor stimulating antibodies (TRAb) in a cohort of hyperthyroid patients and determine their associations to thyroid hormones status, before and after 3 months therapy with carbimazole.

Materials and Methods: Patients were given fixed daily dose of 20 mg carbimazole for 3 months and blood samples were collected at baseline and end of the study. Thirty-eight patients were recruited from the Endocrine Clinic, Hospital Universiti Kebangsaan Malaysia, consisting of 26 females and 12 males, age ranging from 16 to 65 years. Blood samples collected before and at end of study were analysed for TSH, Free T_3, Free T_4, thyroid receptor stimulating antibodies (TRAb), sVCAM-1, sICAM-1 and IL-6.

Results: TRAb level of ≤10 U/L was taken to be negative, while TRAb level of >10 U/L was considered as positive. Twenty-six patients (68%) were TRAb positive (TRAb+) and 12 patients (30%) were TRAb negative (TRAb-). Median TRAb in TRAb+ patients was 23 U/L at baseline, declining to 16.7 U/L ($p<0.001$) in the third 3 month. Serum sVCAM-1 levels were significantly elevated in TRAb+ patients compared to TRAb- (860 versus 499 ng/mL, $p<0.001$). The level decreased significantly to 537 ng/mL with treatment but remained higher than in TRAb- patients ($p=0.003$). Irrespective of TRAb status, all but one patient had elevated serum sICAM-1 levels that remained unaffected by carbimazole therapy. In contrast, IL-6 levels of hyperthyroid patients were within the reference range of 1.4-14.1 pg/mL. Baseline and post-treatment sVCAM-1, and not TRAb levels, were significantly correlated to thyroid hormones.

Conclusion: Compared to other inflammatory markers, sVCAM-1 showed significant correlation to thyroid stimulating antibodies and was most sensitive to changes in thyroid status. The significance of these findings in relation to Graves' disease warrants further investigation.

Key Words: VCAM-1, Graves' disease, TRAb, Inflammation

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Introduction

Thyroid receptor stimulating antibodies (TRAbs) have been shown to be present in about 80 to 95% of patients with Graves' disease (GD).[1,2] Unlike thyroglobulin and thyroid peroxidase antibodies, which can also be found in patients with autoimmune thyroiditis and normal subjects, TRAbs are disease specific. The action of TRAb is analogous to

that of TSH: binding and activating TSH receptors on the surface of thyroid cells, causing increase in intracellular cyclic AMP, thyroid hypertrophy and hyperplasia, and the resultant excess production of thyroxine.[3] In more recent studies, however, adhesion molecules which are involved in cell-to-cell communications leading to cellular events such as the migration of lymphocytes and induction of the autoimmune process, have been postulated to also play significant roles in the pathogenesis of GD. Patients with GD were found to have elevated circulating levels of intercellular adhesion molecule-1 (ICAM-1) and vascular cell adhesion molecule-1 (VCAM-1)[4-8] and the thyrocytes and endothelial cells showed increased expression of the molecules.[9-13] The reason for the increase is not yet clear but is postulated to be either due to the activation of vascular endothelium at non-related sites or a decrease in the metabolism of the adhesion molecules in thyrotoxicosis.

The aim of this study was to determine the levels of VCAM-1, ICAM-1 and cytokine, interleukin-6 (IL-6) in patients with hyperthyroidism, and their associations to TRAb and thyroid hormones status following 3 months of therapy with carbimazole.

Materials and Methods

Subjects

A total of 38 patients were recruited from the Endocrine Clinic, Hospital Universiti Kebangsaan Malaysia, Malaysia. There were 26 females and 12 males, age ranging from 16 to 65 years old, with a mean of 36.5 years. For the purpose of this study, only patients with mild to moderate thyrotoxicosis were recruited; they were either newly diagnosed or if relapsed, has been in remission for at least 6 months. All patients gave their informed consent to participate, and ethical approval for human use was obtained from the relevant body prior to the study. Patients were given carbimazole 20 mg daily and were clinically reviewed every month for 3 months. Blood samples were collected between 8.00 and 10.00 h at the beginning and end of 3 months of therapy. Exclusion criteria included patients with active ophthalmopathy, inflammatory disease, atrial fibrillation, severe thyrotoxicosis, associated ovarian, adrenal, testicular or pituitary disease, renal disease, liver disease, hemolytic anaemia, prostate disease, bone disease, cardiac failure, pericarditis, hypertension and arrhythmias not associated with thyroid disease, or those on drugs such as HRT, beta blockers and steroids.

Assays

Free T_3 (fT_3) and free T_4 (fT_4) were measured using kits purchased from Diagnostic Products Corporation, Los Angeles, CA 90045-5597. The reference ranges quoted for fT_3 and fT_4 were between 2.2-6.8 and 10.3-25.7 pmol/L, respectively. Intra- and interassay coefficient of variations (CVs) for both hormones were within 5% and 7-9%, respectively. Serum TSH was determined by micro particle enzyme immunoassay (MEIA) from Abbott Laboratories, USA. Mean assay sensitivity was 0.04 uIU/mL, and both intra- and interassay CVs were <10%. The reference range for TSH was 0.47-5.0 uIU/mL.

The TRAb kit was purchased from DiaSorin, Stillwater, Minnesota 55082-0285, USA. The assay has been calibrated against the WHO LATS-B (MRC 65/122) and for the purpose of this study, a TRAb level of ≤10 U/L was taken to be negative, while a level of >10 U/L was considered as positive for TRAb. Interassay CV at 12 and 56 U/L was 8.6 and 13% respectively.

The enzyme immunoassay kits for human soluble VCAM-1 (sVCAM-1), soluble ICAM-1 (sICAM-1) and IL-6 ELISA were purchased from MedSystems Diagnostics GmbH, Rennweg 95b, Vienna, Austria. To reduce variability in results, samples collected from each patient were analysed in a single assay.

Statistics

Data were analysed by SAS statistical package, using non-parametric analysis of variance. Differences between groups were compared using Wilcoxon rank sum test and correlation between thyroid hormones and TRAb and adhesion molecules was evaluated by Spearman's Correlation test. Unless otherwise stated, all results are expressed as median and 95% confidence interval (CI). A p value of less than 0.05 was considered as significant.

Results

The overall median and 95% CI levels of thyroid hormones at baseline and at the end of the study are as shown in Table 1. At month 3,7 (18.5%) patients were still hyperthyroid, 16 (42.1%) were subclinically hyperthyroid as indicated by their suppressed TSH but normalised thyroid hormones, 11 (28.9%) become euthyroid, and 4 (10.5%) had actually become hypothyroid.

Data were also analysed according to the TRAb status (Table 2). About 68% (26/38) of patients had TRAb levels above 10 U/L (TRAb+) while the remaining 30% (12/38) were negative (TRAb-). Median TRAb value in TRAb+ patients was 23 U/L at baseline, declining to 16.7 U/L ($p<0.001$) at month 3. Serum sVCAM-1 levels were significantly elevated in TRAb+ patients compared to those who were negative to TRAb (860 versus 499 ng/mL, $p<0.001$). The level decreased significantly to 537 ng/mL with treatment but remained higher than in TRAb- patients ($p=0.003$). In contrast, irrespective of their TRAb status, all but one patient had sICAM-1 level that was significantly higher than the quoted reference range of 115-306 ng/mL. The levels were not affected by carbimazole therapy. However, hyperthyroidism has no effect on IL-6; the levels remained within the reference range of 1.4-14.1 pg/mL and were not affected by carbimazole.

Table 1. Thyroid hormone profile (median and 95 % Confidence Interval) of patients before and after 3 months of carbimazole therapy

	Free T_4 (pmol/L)	Free T_3 (pmol/L)	TSH (mIU/L)
Reference range	10.3 – 26.0	2.2 – 6.8	0.47 – 5.0
Baseline (n = 38)	46.5 (27 – 77)	22.0 (6.3 – 46)	0.01 (0.01 – 0.02)
Month 3 (n = 38)	11.8 (7 – 66)	3.8 (2.4 – 27)	0.1 (0.1 – 8.4)

Numbers represent median (95% CI).

Table 2. Medians (and 95% CI) of thyroid hormones, sVCAM-1, sICAM-1 and IL-6 of TRAb+ and TRAb- patients before and after 3 months of carbimazole therapy

	Visit	TRAb + (>10 U/L, n = 26)	TRAb – (≤10 U/L, n = 12)
Free T_4 (pmol/L)	Baseline	50 (33 - 77)	38 (27 - 56)
	Month 3	12.9 (7 - 66)*	10.7 (7 - 27)*
Free T_3 (pmol/L)	Baseline	26 (6.3 - 46)	12.1 (6.4 - 22.4)
	Month 3	4.6 (2.9 - 27)*	3.5 (2.1 - 8.3)*
TSH (mU/L)	Baseline	0.1 (0.1 - 0.1)	0.1 (0.1 - 0.1)
	Month 3	0.1 (0.1 - 8.4)*	0.8 (0.1 - 5)*
VCAM-1(ng/mL)	Baseline	860 (640 – 1262)	499 (335 - 897)
	Month 3	537 (335 - 886)*†	388 (208 - 606)*
ICAM (ng/mL)	Baseline	798 (356 – 1109)	559 (266- 919)
	Month3	648 (407 - 950)	607 (268 – 997)
IL-6 (pg/mL)	Baseline	1.4(1.4 - 6.2)	1.4 (1.4 –3.5)
	Month 3	1.4 (1.4 – 7)	1.4 (1.4 – 2.4)

Numbers represent median (95% CI).
*p<0.001 versus pre-treatment, † p=0.003 versus respective TRAb- patients.

As expected, fT_3 and fT_4 were significantly higher in TRAb+ patients (p<0.001) but following therapy, the levels decreased to a median value of 12.9 pmol/L which was not significantly different from that obtained in TRAb- patients (10.7 pmol/L, Table 3). Of the 19 patients (73%) who remained positive to TRAb at month 3, 15 (78.9%) were still either hyperthyroid or subclinically hyperthyroid, 2, were (10.5%) in remission while the remainding 2 patients (10.5%) had became hypothyroid.

There was no significant association observed between thyroid hormones and TRAb levels amongst patients with detectable TRAb prior to therapy and in those who remained positive following 3 months of carbimazole. In contrast, sVCAM-1 in TRAb+ patients showed positive correlation to baseline fT_3 (r=0.465, p=0.02, Fig. 1) and the associations were found to be more significant in patients who remained TRAb+ despite therapy (Figs. 2 and 3). No significant correlation was observed between sICAM-1 or IL-6 and the thyroid hormones in either group of patients.

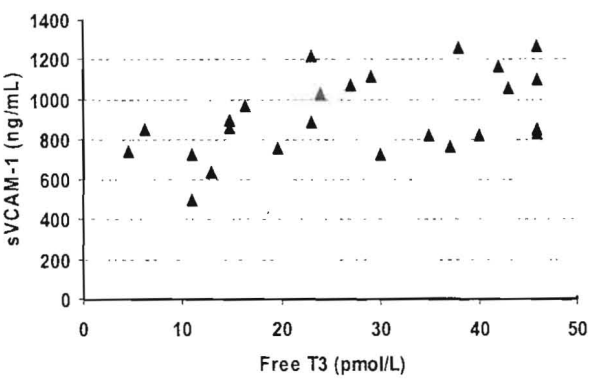

Fig. 1. Correlation between free T_3 and sVCAM-1 in TRAb+ patients before carbimazole therapy

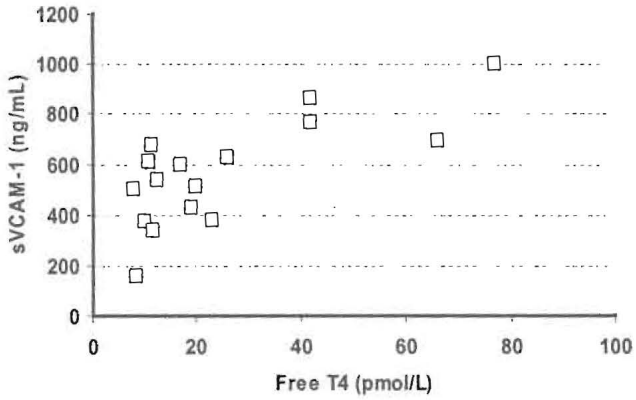

Fig. 2. Correlation between Free T_4 and sVCAM-1 in post-carbimazole TRAb+ patients

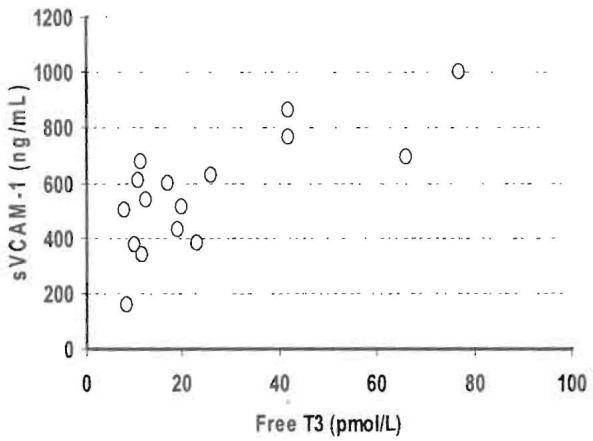

Fig. 3. Correlation between Free T_3 and sVCAM-1 in post- carbimazole TRAb+ patients

Discussion

Several studies have suggested that TRAb measurement is useful in the diagnosis and management of Graves' disease. Presence of TRAb is normally associated with relapse,[14-18] and such patients would benefit from continued treatment.[19,20] Others however, found little predictive value in such measurement and believed that the test should only be carried out when the etiology of the hyperthyroid state could not be determined.[21-23] In those studies, not all patients who relapsed were positive to TRAb and likewise, there were also patients who, despite having presented with detectable TRAb, remained in remission after the antithyroid therapy.[24,25] Similarly in this study, of the 19 patients who remained TRAb+ after 3 months therapy, 2 were euthyroid and another 2 had actually become hypothyroid, thus indicating that the normalization of the thyroid hormones was mainly caused by carbimazole, and whether the patient relapses or remains in remission cannot be attributed solely to the presence or absence of TRAb. On the other hand, such controversy may partly be due to the different assay systems used, whether bioassay or radioreceptor, leading to differences in assay sensitivity.

The significance of adhesion molecules as mediators of events during inflammatory response has been described in many studies. Malignancies and autoimmune disorders including rheumatoid arthritis and Graves' ophthalmopathy were shown to be associated with increased expression of ICAM-1, VCAM-1 and selectins.[7,26,27] ICAM-1 and VCAM-1 are type-1 transmembrane glycoproteins transiently expressed on activated vascular endothelial cells in response to vascular endothelial growth factor and other cytokines, such as TNF-α, interleukin-1β, and interferon.[28] VCAM-1 can be detected in the circulation although the exact mechanism by which it is shredded into the bloodstream is unknown.[29] The markedly elevated sVCAM-1 seen amongst TRAb+ patients was similarly reported by Wenisch et al.[6] Compared to ICAM or IL-6, carbimazole therapy caused significant decrease in sVCAM-1, in parallel to decrease in fT_3 and fT_4, implying, unlike other inflammatory markers, sVCAM-1 is more sensitive to changes in thyroid status

and perhaps, to changes associated with the inflammation process. There is no doubt that more useful data would have been obtained if this study had been continued to include assessment of disease outcome and the significance of sVCAM-1 compared to that of TRAb.

In contrast to several earlier reports,[8,12,26] sICAM-1 remained elevated and was not affected by carbimazole therapy. The absence of any change could probably be explained by the fact that ICAM-1 is more associated with inflammatory activity where levels were reported to increase before the onset of clinical ophthalmology.[26] In this study, active ophthalmopathy was one of the exclusion criteria used. On the other hand, these observations could be due to differences in tissue distributions of the adhesion molecules or variations in the biological half-lives according to disease conditions.[21,30,31] We confirmed the report by Wenisch et al, that thyroid status has no effect on IL-6; the levels remained within the normal range throughout the study duration.[6]

In conclusion, despite its many limitations, this study showed that Graves' disease is indeed a multi-systemic inflammatory disease, affecting not only the thyroid gland but also other tissues such as those associated with immune response and inflammation. Since the usefulness of TRAb as adjunct prognostic marker for Graves' disease is still debatable, it may therefore be worthwhile to further evaluate the clinical significance of sVCAM-1 and its potential use as an alternative marker. Such study should include long-term follow-up and monitoring of patients, and evaluating sVCAM-1 levels in those who remained in remission and comparison with those who later relapsed.

Acknowledgements

The authors wish to acknowledge the Director of the Institute for Medical Research for permission to publish this study, and to Ms Aliah Tawil, Saripah Anom and Kamaruzzaman Hasan for their excellent technical assistance. This study was supported by a research grant funded by the Ministry of Science, Technology and Environment, Malaysia, IRPA grant no: 06-02-03-0720.

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