

Circulating Endothelium-Related Adhesion Molecules in Black South African Patients with Graves' Disease

Vanessa R. Panz,¹ Frederick J. Raal,¹ Jack R. Wall,² and Barry I. Joffe¹

¹Carbohydrate and Lipid Metabolism Research Group, University of the Witwatersrand Medical School, Johannesburg, South Africa; and ²Department of Medicine, Dalhousie University and Queen Elizabeth II Health Sciences Centre, Halifax, Nova Scotia, Canada

Elevated serum concentrations of endothelium-associated adhesion molecules occur in Graves' disease. However, no data exist in African subjects, among whom the incidence is rising. Therefore, 20 black South Africans with Graves' hyperthyroidism were evaluated and 10 healthy controls were also studied. Quantitative determinations of soluble intercellular adhesion molecule-1 (sICAM-1), soluble vascular cell adhesion molecule-1 (sVCAM-1), and soluble E-Selectin (sE-Selectin) were performed in serum samples by an enzyme-linked immunosorbent assay. Mean levels of sVCAM-1 were significantly increased in the thyrotoxic patients compared to controls, but this did not apply to the other adhesion molecules. The presence of ophthalmopathy in 12 patients did not further increase the mean sVCAM-1 concentration, and the administration of antithyroid medication in 5 patients had no measurable effect. In conclusion, sVCAM-1 appears to be a useful marker of active Graves' disease in black South Africans although it does not seem to reflect the occurrence of eye involvement in such patients.

Key Words: Adhesion molecules; Africans; Graves' disease; ophthalmopathy.

Introduction

The incidence of hyperthyroidism owing to Graves' disease is rising among urban black South Africans (1), possibly as a result of exposure to environmental risk factors such as increased dietary iodine ingestion and stress. This may be occurring in other parts of Africa as well (2,3). Thyroid-associated ophthalmopathy is often

observed in this clinical context (4), but its pathogenesis remains unclear. In particular, no close relationship has been noted between antiflavoprotein antibodies or thyroid-stimulating hormone (TSH)-receptor antibodies and ocular involvement in black South African patients (5).

Leukocyte adhesion and signaling are important steps in the development of immune and inflammatory responses (6). The migration of leukocytes to the vascular endothelium during the inflammatory process is mediated by the expression of certain adhesion molecules. They include soluble intercellular adhesion molecule-1 (sICAM-1), soluble vascular cell adhesion molecule-1 (sVCAM-1), and soluble E-Selectin (sE-Selectin). In previous studies, markedly elevated serum concentrations of these endothelium-associated adhesion molecules have been found in untreated patients with Graves' disease (7–9). However, there is a lack of data in African patients, and therefore this study was undertaken to examine the potential role of adhesion molecules in the pathogenesis of Graves' disease in black South Africans.

Results

Demographic and biochemical data of the patients with Graves' disease and normal subjects are summarized in Table 1. As expected, levels of free thyroxine (FT₄) were significantly increased ($p < 0.00001$) and TSH concentrations were significantly decreased ($p < 0.00001$) in the thyrotoxic patients compared with normal subjects. Mean levels of serum sICAM-1 and sE-Selectin were not significantly different between patients and controls. However, thyrotoxic patients showed a significantly increased mean sVCAM-1 concentration compared with normal subjects ($p < 0.001$). No significant correlation was found between serum values of the individual adhesion molecules and FT₄ concentrations.

When the 5 patients who had been on carbimazole were analyzed as a separate cohort, their mean levels of circulating adhesion molecules were found to be similar to the 15 untreated subjects (Table 2), despite lower FT₄ values. Similarly, when the 12 patients with clinical ophthalmopathy were evaluated

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Author to whom all correspondence and reprint requests should be addressed: Dr. Vanessa R. Panz, Carbohydrate and Lipid Metabolism Research Group, University of the Witwatersrand Medical School, 7 York Road, Parktown 2193, Johannesburg, South Africa. E-mail: 014panz@chiron.wits.ac.za.

Table 1
Demographic and Biochemical Data in Black Hyperthyroid Patients and Normal Subjects ^a

	Hyperthyroid patients	Normal subjects
Number of subjects	20	10
Gender (M/F)	2/18	2/8
Age (yr)	38 ± 10	42 ± 7
sICAM-1 (ng/mL)	355 ± 299	238 ± 38
sVCAM-1 (ng/mL)	986 ± 307 ^b	615 ± 140
sE-Selectin (ng/mL)	86 ± 39	60 ± 17
FT ₄ (pmol/L)	80 ± 49 ^c	13 ± 1
TSH (mIU/L)	0.02 ± 0.004 ^c	1.4 ± 0.4

^a Values are expressed as means ± SD.

^b Significance of values in the patient group compared with normal subjects: $p < 0.001$.

^c Significance of values in the patient group compared with normal subjects: $p < 0.00001$.

as a separate subgroup, their mean levels of serum adhesion molecules were not significantly different from the 8 subjects without this complication (data not shown).

Discussion

Graves' disease in African patients, as is the case with other populations and ethnic groups, has an autoimmune etiology, with TSH-receptor antibodies being detected in the majority of cases (4). While ophthalmopathy is often present, it is not closely related to other autoimmune markers (5). However, several studies have reported raised serum concentrations of soluble endothelium-associated adhesion molecules in patients with Graves' disease in general (7–9), and in those with ophthalmopathy in particular (10), in which they appear to be useful markers of disease activity and evolving eye disease. It was in the context of this background that our present study was conducted.

Mean serum levels of all three soluble adhesion molecules tended to be increased in black subjects with Graves' disease, although only sVCAM-1 reached statistical significance. Since VCAM-1 binds functionally to very late activation antigen-4, expressed on all leukocytes except neutrophils (11,12), this may reflect the activation of specific components or stages of the inflammatory process. However, the number of patients tested was relatively small, and the administration of antithyroid medication (albeit for a relatively short period) may have altered the concentration of the other adhesion receptors measured (13), although our own data did not support this contention. Unlike in previous studies (9,13), the presence of clinical ophthalmopathy in our patients was not associated with a further increment in cell adhesion molecule concentrations. However, the lack of orbital radiology makes it difficult to draw definitive conclusions about the role of these cell-surface proteins in the pathogenesis of ocular involvement in African thyrotoxic patients, since this complication is not

Table 2
Comparison of Circulating Endothelium-Related Adhesion Molecules in Black Hyperthyroid Patients With and Without Therapy ^a

	With therapy	Without therapy
Number of patients	5	15
sICAM-1 (ng/mL)	414 ± 253	335 ± 177
sVCAM-1 (ng/mL)	916 ± 246	1009 ± 329
sE-Selectin (ng/mL)	88 ± 32	86 ± 42

^a Values are expressed as means ± SD.

reliably detected on clinical grounds alone. Further studies incorporating orbital imaging are required for greater clarity on this issue.

Patients and Methods

Patients

Twenty black South African patients (18 females and 2 males, aged 17–55 yr) with hyperthyroidism owing to Graves' disease were studied. They were all recently diagnosed, although five patients had been started on anti-thyroid medication (carbimazole) for less than 3 mo. The diagnosis of hyperthyroidism was made from the usual clinical criteria and confirmed by demonstrating elevated levels of serum FT₄ and suppressed TSH. An isotopic thyroid scan showed rapid and diffuse uptake of technetium in all patients. None of the patients suffered from infections, allergies, or other autoimmune diseases. Ophthalmopathy, detected by careful clinical observation and classified according to the recommendations of a committee of the International Thyroid Associations (14), was documented in 12 cases. Orbital imaging studies were not performed because these are not readily available at our hospital. As a control group, 10 healthy age- and sex-matched black South Africans (9 females and 1 male, aged 26–50 yr) were studied. They had no clinical or biochemical evidence of thyroid dysfunction or ophthalmopathy.

Study Design

Each patient was assessed clinically at the same time as blood samples were taken for the measurement of thyroid hormones and adhesion molecules. Sera from patients and normal subjects were stored at –70°C until analyzed. The study was approved by the Committee for Research on Human Subjects of the University of the Witwatersrand.

Measurement of Thyroid Hormones

Serum concentrations of FT₄ and TSH were measured using commercial kits (Chiron Diagnostics Automated Chemiluminescence System, Boston, MA). The interassay coefficient of variation (CV) was 6.6% for FT₄ (normal range: 11.5–23.2 pmol/L) and 6.3% for TSH (normal range: 0.35–5.50 mIU/L).

Measurement of Adhesion Molecules

Quantitative determinations of sICAM-1, sVCAM-1, and sE-Selectin were performed in serum samples by a sandwich enzyme-linked immunosorbent assay using kits supplied by R & D Systems. Interassay CVs were 10.8% for sICAM-1 (normal range: 115–306 ng/mL), 6.7% for sVCAM-1 (normal range: 395–714 ng/mL), and 8.1% for E-Selectin (normal range: 29.1–63.4 ng/mL). Analysis of the samples was done without reference to the clinical findings.

Statistical Analysis

Distribution of the data was nonparametric and the Wilcoxon Rank Sum Test was used to evaluate the differences between the patients and normal subjects. A level of $p < 0.05$ was considered statistically significant. Values are expressed as means \pm SD. Levels of the individual adhesion molecules were correlated by the Spearman's test to FT₄ concentrations in all patients.

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