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Thyroid-Associated Ophthalmopathy in Black South Africans with Graves' Disease

Relationship to Serum Antibodies Reactive Against Eye Muscle and Orbital Connective Tissue Autoantigens

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The prevalence of hyperthyroidism owing to Graves' disease is increasing among urban black South Africans. Thyroid-associated ophthalmopathy is often observed in this context, but its pathogenesis remains unclear. No close relationship has been noted between antiflavoprotein (Fp) antibodies or thyrotropin receptor antibodies and ocular involvement in black patients. We measured serum antibodies against eye muscle and orbital connective tissue antigens in black patients with Graves' disease, correlating them with eye signs. Of 11 patients with clinical ophthalmopathy, 2 (18%) had antibodies against collagen type XIII, 3 (27%) against flavine adenine dinucleotide (FAD), 1 (9%) against Fp, and 4 (35%) against G2s. Antibody prevalences in eight patients without clinical ophthalmopathy were 12.5% for collagen XIII, 12.5% for FAD, 25% for Fp and 0% for G2s. These differences were not statistically significant. None of the individual mean antibody levels were significantly different between the two subgroups of thyrotoxic patients. Serum antibody levels were negative in 10 black South African controls. In summary, eye muscle and orbital connective tissue antibodies were found in small proportions of patients with Graves' disease with no close relationship of any antibody to eye signs. Thus, a substantial proportion of black South Africans with overt clinical ophthalmopathy remains in whom currently availabe serologic tests are unhelpful for screening and laboratory confirmation.

Key Words: Graves' disease; ophthalmopathy; flavoprotein; G2s; collagen XIII.

Introduction

The prevalence of hyperthyroidism owing to Graves' disease is increasing among urban black South Africans, irrespective of better health care facilities for this portion of the population *(1)*. This may be occurring in the rest of Africa as well *(2,3)*. Thyroid-associated ophthalmopathy (TAO) is often observed in this clinical context *(4),* but its pathogenesis remains unclear. In particular, no close relationship has been noted among antiflavoprotein antibodies, thyrotropin (thyroid-stimulating hormone [TSH])-receptor antibodies, or circulating adhesion molecules and ocular involvement in black South African patients *(5,6)*.

We have recently identified a series of novel serum antibodies reactive against several eye muscle and orbital connective tissue antigens in patients with TAO. These include the so-called 64-kDa protein *(7,8)*, now shown to be the flavoprotein (Fp) subunit of mitochondrial succinate dehydrogenase *(9,10)*; flavine adenine dinucleotide (FAD), a cofactor used by succinate dehydrogenase; a novel thyroid and eye muscle shared protein, G2s *(11)*; and type XIII collagen. Type XIII collagen was recently cloned by Pihlajaniemi and Rehn *(12)* and Hagg et al. *(13)* and shown to be expressed in the plasma membrane fraction, where it could be the target of an autoimmune reaction in connective tissue disorders. Through collaboration with these investigators, we have obtained preliminary evidence for autoantibodies against collagen type XIII in patients with TAO, particularly those with the congestive ophthalmopathy subtype *(14)*. Such antibodies appear to be markers of congestive ophthalmopathy and may contribute to the inflammatory reaction and progression of orbital disease. In the present study, we have reevaluated the potential role of autoimmunity against these orbital autoantigens in the diagnosis of ophthalmopathy in black South Africans with Graves' disease. We failed to show any close relationship between such antibodies and eye signs, suggesting that other diagnostic markers should be sought in these patients.

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Table 1
Clinical, Biochemical, and Immunologic Data in Black South African Hyperthyroid Patients With and Without Ophthalmopathy[a]

Patient	Age (yr)	Sex	Ophthalmopathy	FT_4 (pmol/L)	TSH (mIU/L)	Collagen XIII[b]	FAD[c]	Fp[d]	G2s[e]
3	54	F	Yes (mild)	100	<0.03	0.249[a]	0.405[a]	0.105	0.238[a]
4	43	F	Yes (gross)	25.7	<0.03	0.292[a]	0.261[a]	0.204	0.187[a]
5	55	F	Yes (gross)	43.4	<0.03	0.152	0.154	0.074	0.079
8	18	F	Yes (mild)	167	<0.03	0.096	0.11	0.072	0.067
10	44	F	Yes (gross)	32.3	<0.03	0.109	0.073	0.152	0.052
11	33	F	Yes (gross)	151	<0.03	0.179	0.165	0.156	0.087
12	40	F	Yes (mild)	64.3	<0.03	0.172	0.231[a]	0.236[a]	0.171[a]
14	44	M	Yes (mild)	100	<0.03	0.102	0.179	0.111	0.045
15	28	F	Yes (gross)	96	<0.03	—	—	—	—
18	46	F	Yes (mild)	37.0	<0.03	0.15	0.21	0.132	0.085
19	30	F	Yes (mild)	26.1	<0.03	0.175	0.196	0.137	0.145[a]
20	38	F	Yes (gross)	76.1	0.04	0.114	0.123	0.131	0.12
Mean	39.4			76.6		0.163	0.192	0.125	0.116
SD	10.8			47.8		0.062	0.089	0.065	0.062
1	28	M	No	46.5	<0.03	0.295[a]	0.032	0.064	0.039
2	29	F	No	60.2	<0.03	0.196	0.104	0.195	0.149
6	29	F	No	15.1	<0.03	0.17	0.159	0.202	0.139
7	35	F	No	154	<0.03	0.036	0.077	0.072	0.013
9	22	F	No	144	<0.03	0.212	0.17	0.271[a]	0.077
13	42	F	No	30.3	<0.03	0.075	0.26[a]	0.058	0.113
16	39	F	No	130	<0.03	0.117	0.073	0.475[a]	0.078
17	45	F	No	111	<0.03	0.208	0.192	0.184	0.127
Mean	33.6			86.4		0.164	0.133	0.190	0.092
SD	7.9			54.6		0.084	0.095	0.139	0.049

[a]Denotes positive antibody test (OD > mean + 2 SDs above control subjects).
[b]Collagen XIII upper limit of normal: 0.223 for patients 1–13; 0.309 for patients 14–20.
[c]FAD upper limit of normal: 0.175 for patients 1–13; 0.285 for patients 14–20.
[d]Fp upper limit of normal: 0.223 for patients 1–13; 0.325 for patients 14–20.
[e]G2s upper limit of normal: 0.165 for patients 1–13; 0.145 for patients 14–20.

Results

Table 1 summarizes the clinical, biochemical, and immunologic data on all 20 black South African patients with Graves' disease. As expected, all subjects showed markedly suppressed TSH concentrations, whereas their serum-free thyroxine (FT_4) levels (with one exception) were elevated. We measured serum antibodies against eye muscle and orbital connective tissue antigens, correlating them with eye signs. Of the 11 patients with clinically documented ophthalmopathy in whom antibody tests were performed, 2 (18%) had antibodies against collagen XIII, 3 (27%) had antibodies against FAD, only 1 (9%) tested positive against Fp, and 4 (35%) had antibodies against G2s. Corresponding antibody prevalences in the eight patients without clinical ophthalmopathy were 12.5% for collagen XIII, 12.5% for FAD, 25% for Fp, and 0% for G2s. None of these differences were statistically significant on Fisher's exact analysis (p = NS). Similarly, none of the individual mean antibody levels, expressed as optical density (OD), were significantly different between the two subgroups of thyrotoxic South African patients (unpaired t-test, p = NS). There were no positive correlations between any of these antibodies and eye signs, although only one patient had clinical evidence of ocular myopathy (Fig. 1). Serum antibody levels were uniformly negative in the 10 control subjects tested.

Discussion

TAO appears to consist of two clinical subtypes: ocular myopathy and congestive ophthalmopathy *(10,15)*. A previous investigation among black South Africans suggested that although ocular involvement is often observed, it appears to be mainly of the congestive variety *(5)*. It was hoped, therefore, that the present study, using an array of novel serum eye muscle and orbital connective tissue antibodies, would allow this distinction to be confirmed. Type XIII collagen is an important autoantigen in congestive ophthalmopathy *(14)*. Yet, positive antibodies against this connective tissue antigen were only found in two of our patients with congestive ophthalmopathy (mainly consist-

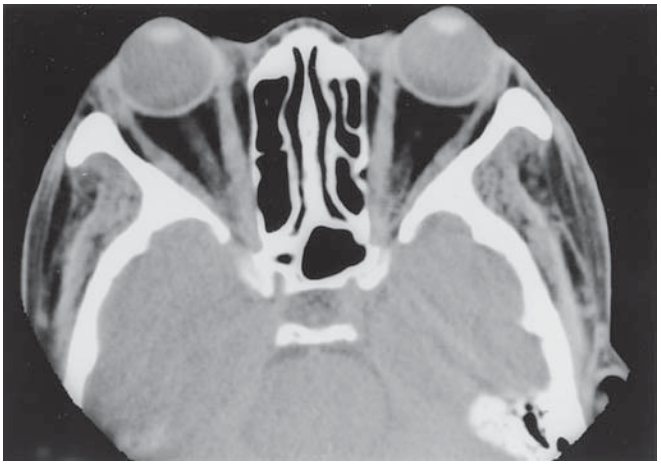

Fig. 1. CAT scan of the orbit, demonstrating enlargement of the extraocular muscles seen in patient no. 4.

ing of lid-lag, proptosis, and chemosis), including one who had extraocular muscle involvement as well. Moreover, antibodies were also detected in one hyperthyroid individual with no eye involvement. However, it is conceivable that this patient was destined to develop ocular signs later in his clinical course, because no follow-up observations were made. A somewhat more sensitive and specific marker associated with ophthalmopathy in our study were autoantibodies against the G2s fusion protein. Recent findings *(11)* suggest that this novel eye muscle and thyroid shared protein may explain the link between ophthalmopathy and Graves' hyperthyroidism in different ethnic groups.

Certain limitations of our study warrant consideration. Because orbital radiology was not routinely performed, ocular myopathy might have been missed in some of the patients. However, this probably does not influence the interpretation of our findings, because we still failed to detect any serum antibodies in the majority of cases in which ophthalmopathy was already clinically apparent. Similarly, the relatively small number of thyrotoxic subjects investigated and lack of follow-up data hamper the wider extrapolation of our observations to the rest of the African continent, and they should be considered of a somewhat preliminary nature, awaiting confirmation in a larger, multinational study.

Nevertheless, there remains a substantial proportion of black South Africans with overt clinical ophthalmopathy in whom currently available serologic tests are clearly not helpful for screening and laboratory confirmation. The reasons for this are uncertain but might reflect a tendency for impaired autoantibody production in various autoimmune endocrine disorders, a suggestion supported by our recent observation of a relatively reduced prevalence of serum autoantibodies to glutamic acid decarboxylase in black South Africans with type 1 diabetes mellitus *(16)*. Therefore, further immunologic studies are required to elucidate the pathogenesis of ocular damage in this group of patients.

Materials and Methods

Patients

Twenty black South African patients (18 females and 2 males, ages 17–55) with hyperthyroidism owing to Graves' disease were studied consecutively. They were all recently diagnosed, although five patients had been started on antithyroid medication (carbimazole) less than 3 mo previously. The diagnosis of hyperthyroidism was made from the usual clinical criteria and confirmed by demonstrating elevated levels of FT_4 and suppressed thyrotropin (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 *(17)*, was documented in 12 cases at the time of the initial assessment. This consisted of lid-lag and stare, proptosis, and chemosis in all patients; one subject (patient no. 4) also had extraocular muscle involvement, confirmed by computed axial tomography (CAT) scan of the orbit (Fig. 1). However, orbital imaging studies were not routinely performed unless clinically indicated. As a control group, 10 healthy age- and sex-matched black South Africans (9 females and 1 male, ages 26–50) were studied. They had no clinical or biochemical evidence of thyroid dysfunction or ophthalmopathy. Informed consent was obtained from all patients and control subjects who participated in the project.

Study Design

Each patient was assessed clinically at the same time that blood samples were taken for the measurement of thyroid hormones and orbital antibodies. Sera from patients and normal subjects were stored at –70°C until air-freighted to Halifax, Canada, at the end of the study. The study was approved by the Committee for Research on Human Subjects of the University of the Witwatersrand. Analysis of serum samples was performed blind; that is, antibody tests were carried out in Halifax at the end of the study without reference to the clinical findings.

Measurement of Thyroid Hormones

Serum concentrations of FT_4 and TSH were measured using commercial kits (Chiron Diagnostics Automated Chemiluminescence System, Boston, MA). The interassay coefficient of variation was 6.6% for FT_4 (normal range: 11.5–23.2 pmol/L) and 6.3% for TSH (normal range: 0.35–5.50 mIU/L).

Measurement of Serum Muscle Antibodies

A standard enzyme-linked immunosorbent assay method, as described previously *(18,19)*, was employed to measure the various serum eye muscle antibody titers. The concentrations of antigens used in these assays were as

follows: 0.125 μg/mL of type XIII collagen, 0.25 μg/mL of FAD, 0.125 μg/mL of Fp, and 0.25 μg/mL of G2s. Recombinant human type XIII collagen was obtained from Dr. T. Pihlajaniemi, (University of Ouulu, Finland). FAD was purchased from Sigma-Aldrich (St. Louis, MO), and the Fp subunit of mitochondrial succinate dehydrogenase was purified from beef heart muscle as described previously *(9,10)*. G2s fusion protein was purified using a pFLAG-ATS Escherichia coli expression vector (Sigma-Aldrich) as previously described *(11)*. Serum dilution was 1/25 and that of the second antibody 1/2000, for all antigens. Results were expressed as OD at 405 nm and a positive test defined as OD > mean + 2 SDs for a panel of 10 age- and sex-matched normal subjects, the upper limit of normal.

Statistical Analyses

We used the unpaired *t*-test for analysis of mean (±SD) FT_4 and antibody values between groups. We compared the prevalence of positive eye muscle antibodies in patients with and without ophthalmopathy by Fisher's exact test. Statistical differences were considered significant at $p < 0.05$.

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References

1. Kalk, W. J. (1981). *East Afr. Med. J.* **58,** 109–116.
2. Mengistu, M. (1993). *Ethiop. Med. J.* **3,** 25–36.
3. Modebe, O. (1995). *Afr. J. Med. Med. Sci.* **24,** 347–351.
4. Zouvanis, M., Panz, V. R., Kalk, W. J., and Joffe, B. I. (1998). *J. Endocrinol. Invest.* **21,** 771–774.
5. Joffe, B. I., Gunji, K., Panz, V., Ackrell, B., et al. (1998). *Thyroid* **8,** 1023–1027.
6. Panz, V. R., Raal, F. J., Wall, J. R., and Joffe, B. I. (2000). *Endocrine* **12,** 21–24.
7. Salvi, M., Miller, A., and Wall, J. R. (1988). *FEBS Lett.* **232,** 135–139.
8. Wu, Y. I., Clarke, E. M., and Shepherd, P. (1998). *Thyroid* **8,** 167–174.
9. Kubota, S., Gunji, K., Ackrell B. A., et al. (1998). *J. Clin. Endocrinol. Metab.* **83,** 443–447.
10. Gunji, K., De Bellis, A., Kubota, S., et al. (1999). *J. Clin. Endocrinol. Metab.* **84,** 1255–1262.
11. Gunji, K., De Bellis, A., Li, A. W., et al. (2000). *J. Clin. Endocrinol. Metab*., **85,** 1641–1647.
12. Pihlajaniemi, T. and Rehn, M. (1995). *Prog. Nucleic Acid Res. Mol. Biol*. **50,** 225–262.
13. Hagg, P., Rehn, M., Huhtala, P., Vaisanen, T., Tamminen, M., and Pihlajaniemi, T. (1998). *J. Biol. Chem.* **273,** 15,590–15,597.
14. Wall, J. R., Blanchard, M. E., West, K., and Pihlajaniemi, T. (1999). Paper presented at the 72nd Annual meeting of the American Thyroid Association (abstract), Palm Springs, FL.
15. Soloyeva, T. P. (1989). *Orbit* **3,** 193–198.
16. Panz, V. R., Kalk, W. J., Zouvanis, M., and Joffe, B. I. (2000). *Diabetic Med.,* **17,** 524–527.
17. Committees of the American, European, Asia-Oceania, and Latin America Thyroid Associations. (1992). *Thyroid* **2,** 235, 236.
18. Miller, A., Sikorska, H., Salvi, M., and Wall, J. R. (1986). *Acta Endocrinol*. **113,** 514–522.
19. Kapusta, M., Salvi, M., Triller, H., Gardini, E., Bernard, N., and Wall, J. R. (1990). *Autoimmunity* **7,** 33–40.