Anti-eye Muscle IgG and IgM Antibodies are Associated with Eye Muscle Type 2 Deiodinase Activities in Hyperthyroid Graves’ Ophthalmopathy

Ildikó Molnár and Éva Somogyiné-Vári

Abstract

Objective: Antibodies to eye muscle tissue are demonstrated in hyperthyroid Graves’ disease but their pathognomonic roles have not been clarified yet. We suggest that type 2 deiodinase (DIO2) enzyme can be a target between orbital and thyroid diseases in hyperthyroid Graves’ ophthalmopathy. In this study, the relationship between anti-eye muscle membrane (EyeM) and cytosol (EyeC) IgG, IgA and IgM antibodies and eye muscle DIO2 activity was investigated in hyperthyroid Graves’ disease.

Methods: Thirty-two patients with hyperthyroid Graves’ disease (20 had ophthalmopathy), mean age 36 ± 13 years, 27 females and 5 males formed the patient group. Thyroid hormone levels were measured with chemiluminescence immunoassay, but TSH receptor antibodies with radioimmunoassay. Eye muscle DIO2 activity was measured using 125I-T3.

Results: EyeM and EyeC IgG antibodies were associated with increased eye muscle DIO2 activity compared to those who were negative for these antibodies (2.79 ± 2.53 vs 17.88 ± 20.06 pmol/mg/min, P<0.015 for EyeM-IgG and 6.88 ± 9.62 vs 26.76 ± 23.86 pmol/mg/min, P<0.008 for EyeC-IgG). The difference in EyeC-IgM antibodies was significant between patients with and without ophthalmopathy (10.1 ± 4.73 vs 3.46 ± 3.18 pmol/mg/min, P<0.023). The presence of EyeM-IgM antibodies was associated with lower eye muscle DIO2 activities with significantly decreased eye muscle thicknesses (8.51 ± 15.38 vs 2.6 ± 0.1 pmol/mg/min and 4.71 ± 1.23 vs 3.9 ± 0.14 mm, P<0.04).

Conclusion: Anti-eye muscle antibodies were associated with increased or decreased eye muscle DIO2 activities affecting eye muscle thicknesses in hyperthyroid Graves’ ophthalmopathy. DIO2 enzyme can be a new target between orbital and thyroid diseases in hyperthyroid Graves’ ophthalmopathy.

Keywords: Anti-eye muscle antibodies; Type 2 deiodinase; Hyperthyroid Graves’ disease; Graves’ ophthalmopathy

Introduction

Graves’ ophthalmopathy is an organ-specific autoimmune disease with the metabolic feature of hyperthyroidism, diffuse goitre, and asymmetrical increased volume of orbital tissues due to local inflammatory and immune processes [1]. Several antibodies to orbital tissues were demonstrated highlighting their clinical importance but their pathognomonic roles have not been confirmed [2-4]. We demonstrated the binding features of IgG, IgA and IgM isotype anti-eye muscle membrane and cytosol antibodies to different molecular weights of eye muscle proteins [5]. A clinical relevance was found between proptosis and the presence of anti-eye muscle membrane IgA antibodies [6]. The increase of retrobulbar fat volume and eye muscle enlargements lead to proptosis, diplopia and rarely sight loss due to optic nerve compression [7]. Eye muscle enlargements are very frequent symptom of Graves’ ophthalmopathy and affect differently extraocular muscles, such as medial, lateral, superior and inferior muscles [8].
Patients and Methods

Patients

Thirty two hyperthyroid patients with Graves' disease, 20 of whom had ophthalmopathy, mean age of 36 ± 13 years, 27 females and 5 males formed the patient group. The disease duration was 1 ± 2 months. Thyroid hormone serum levels were as follows: 0.047 ± 0.07 mIU/l for TSH, 4.438 ± 42.77 pmol/l for FT4, 11.07 ± 6.74 pg/ml for FT3. Antithyroid antibody serum levels were as follows: 58.9 ± 95.2 IU/l for TSH receptor antibodies, 1.95 ± 1.3 in ELISA index for thyroid peroxidase antibodies, 1.26 ± 0.44 in ELISA index for thyroglobulin antibodies. Hyperthyroidism was diagnosed by supprimated TSH levels with increased FT3 and FT4 levels. Ultrasonography was carried out by radiologist in all cases to measure extraocular muscle thicknesses. Toxic goitre was excluded using thyroid Technetium-99m scintigraphy and ultrasonograph. NOSPECS evaluation was applied for the classification of ophthalmopathy [13]. All patients were active for ophthalmopathy, 18 patients had soft tissue involvement, 14 patients had proptosis and 10 patients had eye muscle enlargements.

None of the patients had corneal ulcer and/or optic nerve compression. Twenty-seven patients were untreated in the time of the study, and only 5 patients with Graves' ophthalmopathy received methimazole (15-30 mg/day) treatment with propranolol (20-40 mg/day).

Methods

Detection of anti-eye muscle membrane (EyeM) and cytosol (EyeC) antibodies: Antibodies against eye muscle membrane (EyeM) and cytosol (EyeC) fractions were measured by enzyme-linked immunosorbent assay (ELISA) detailed in our previous paper [14]. EyeM and EyeC fractions were prepared by centrifugations. The pellet of 100,000 x g was served as membrane fraction and the supernatant as cytosol fraction. Eye muscle tissue were removed within 4-6 h after death from patients who did not suffer from tumor, endocrine and infectious diseases. The 96-well plates were pretreated with 50 μg/ml EyeM or EyeC fraction per wells and incubated overnight at 4°C. Patient sera of 100 μl (in dilution of 1:100) were added to each well for 2 h at room temperature. Goat anti-human IgG, IgA and IgM antibodies were conjugated with horseradish peroxidase (in dilution of 1:2000) (SIGMA, USA) and the proteins were precipitated by adding 500 μl 20% ethylenediaminetetraacetic acid (EDTA, Sigma, USA) in a volume of 250 μl was prepared at ice-cold temperature. The reaction was induced by putting the mixture at 37°C for 90 min. The reaction was stopped by adding 100 μl 5% ice-cold bovin serum albumin (BSA, Sigma, USA) and the proteins were precipitated by adding 500 μl 20% trichloroacetic acid (Reanal, Hungary). The radioactivity of acid-soluble radioiodine samples and the blank (saline instead of serum, represented the total activity) were measured in gamma counter (Gamma NZ 322, Hungary). The samples were used in duplicates. DIO2 activity was expressed as pmol of T4 converted per mg/min protein. Elevated T3 serum levels in hyperthyroid patient sera may have a substrate inhibitory effect on DIO3 activities. The results were extrapolated at 1 pmol/l of T4.

Detection of thyroid hormone and anti-thyroid autoantibody levels

Thyroid hormone levels (TSH, FT4, FT3) were measured with chemiluminescence immunoassay (LIA-MAT, Byk Sangtec, Germany) and antithyroid antibodies to thyroid peroxidase (TPO) and thyroglobulin (Htg) with ELISA method. TSH receptor antibodies were tested with radioimmunoassay (Brahms, Germany). The normal values of thyroid hormones were as follows: 7.72-23.18 pmol/l for FT4, 2.5-4.5 pg/ml for FT3 and 0.3-3.0 mIU/l for TSH. Thyroid peroxidase and thyroglobulin were used in concentrations of 50 μg/ml after the preparation procedure described in detail in our previous paper [16]. ELISA tests for the detection of anti-TPO and anti-Htg antibodies were similar to the detection procedure of anti-eye muscle antibodies made by hospital laboratory and the results were also displayed in ELISA index: the ratio of patient sample O.D. and mean O.D. of controls (healthy subjects, n=20). The cut-off values were as follows: 1.86 for anti-Htg antibodies and 1.9 for anti-TPO antibodies. TSH receptor antibody values above 14 IU/l were regarded as positivity.

Statistical analysis

The data were displayed as mean ± SD, but the results were exhibited as mean ± standard error (SE) in Figures. Chi-squared statistical test with Yates's correction and Student's t-test were applied for comparison of two groups. Pearson's correlation was carried out to demonstrate any association between anti-eye muscle antibodies and eye muscle DIO2 activities.

Results

Occurrence of anti-eye muscle membrane (EyeM) and cytosol (EyeC) antibodies in hyperthyroid Graves’ disease

The presence of EyeM and EyeC IgG, IgM, but not IgA antibodies could be demonstrated in hyperthyroid patients with Graves’ disease (Table 1). In hyperthyroid Graves’ ophthalmopathy, EyeM-IgG antibodies were found in 4 out of 13 cases, EyeM-IgM in 2 out of 14 cases, EyeC-IgG in 1 out of 19 cases and EyeC-IgM in 8 out of 19 cases. In hyperthyroid Graves’ disease without ophthalmopathy, EyeM-IgG antibodies were found in 2 out of 6 cases, EyeC-IgG in 1 out of 11 cases and EyeC-IgM in 3 out of 11 cases. EyeM-IgM antibodies were detected only in Graves’ ophthalmopathy and the difference was significant between the patients with and without ophthalmopathy (P<0.005).
Occurrence of anti-eye muscle antibodies

| Anti-eye muscle antibodies | Graves’ disease without ophthalmopathy (n=12) | Graves’ disease with ophthalmopathy (n=20) | N | P |
|---------------------------|---------------------------------------------|-------------------------------------------|---|---|
|                           | No                                      | Yes                                      |    |   |
| EyeM-IgG                  | 4                                       | 2                                        | 11 | 4  | 6  | 0.005* |
| EyeM-IgA                  | 0                                       | 0                                        | 0  | 0  |    |      |
| EyeM-IgM                  | 6                                       | 0                                        | 13 | 2  | 2  |      |
| EyeC-IgG                  | 10                                      | 1                                        | 18 | 1  | 1  |      |
| EyeC-IgA                  | 0                                       | 0                                        | 0  | 0  |    |      |
| EyeC-IgM                  | 8                                       | 3                                        | 11 | 8  | 11 |      |

EyeM: Eye membrane fraction; EyeC: Eye muscle cytosol fraction; *Chi-squared statistical test with Yates’s correction

Table 1: Occurrence of anti-eye muscle membrane (EyeM) and cytosol (EyeC) IgG, IgA and IgM antibodies in hyperthyroid Graves’ disease.

Figure 1: Anti-eye muscle membrane (EyeM) and cytosol (EyeC) IgG antibodies were associated with increased eye muscle DIO2 activities in hyperthyroid Graves’ disease.

Anti-eye muscle antibodies were associated with increased or decreased eye muscle DIO2 activities in hyperthyroid Graves’ disease

The presence of EyeM-IgG and EyeC-IgG antibodies was associated with significantly increased eye muscle DIO2 activities compared to those who were negative for these antibodies in hyperthyroid Graves’ patients (2.79 ± 2.53 vs 17.88 ± 20.06 pmol/mg/min, P<0.015 for EyeM-IgG and 6.88 ± 9.62 vs 23.86 pmol/mg/min, P<0.008 for EyeC-IgG) (Figure 1). EyeM-IgG and EyeC-IgG antibodies correlated positively with eye muscle DIO2 activities (r=0.8124, P<0.0001) (Figure 1). However, the presence of EyeM-IgM and EyeC-IgM antibodies was associated with decreased eye muscle DIO2 activities. The differences in eye muscle DIO2 activities between the presence and absence of EyeM-IgM and EyeC-IgM antibodies were not significant (7.57 ± 12.98 vs 2.6 ± 0.99 pmol/mg/min for EyeM-IgM and 9.8 ± 13.83 vs 5.27 ± 4.6 pmol/mg/min for EyeC-IgM).

Figure 2: Anti-eye muscle membrane (EyeM) and cytosol (EyeC) IgG antibodies correlated positively with eye muscle DIO2 activities in hyperthyroid Graves’ disease. EyeM: Eye muscle membrane, EyeC: Eye muscle cytosol.

Relationship among eye muscle DIO2 activities, eye muscle DIO2 activities and eye muscle thicknesses in hyperthyroid Graves’ ophthalmopathy

In hyperthyroid Graves’ ophthalmopathy, the presence of EyeM-IgG antibodies was associated with significantly increased eye muscle DIO2 activities but only relevant eye muscle enlargements in comparison with those who were negative for these antibodies (2.45 ± 1.83 vs 22.21 ± 23.96 pmol/mg/min, P<0.012 and 4.32 ± 0.53 vs 5.6 ± 2.35 mm) (Figure 1). However, the presence of EyeM-IgM antibodies was associated with relevant decreased eye muscle DIO2 activities with significantly decreased eye muscle thicknesses (8.51 ± 15.38 vs 2.6 ± 0.1 pmol/mg/min and 4.71 ± 1.23 vs 3.9 ± 0.14 mm, P<0.04). The difference in EyeC-IgM antibodies was significant between patients with and without ophthalmopathy. The presence of EyeC-IgM antibodies was associated with significantly lower eye muscle DIO2 activities in hyperthyroid Graves’ patients with ophthalmopathy compared to those who did not have ophthalmopathy (10.1 ± 4.73 vs 3.46 ± 3.18 pmol/mg/min, P<0.023) (Figure 2).
Figure 3: Anti-eye muscle membrane (EyeM) IgG antibodies were associated with increased, but IgM antibodies with decreased eye muscle DIO2 activities and eye muscle thicknesses in hyperthyroid Graves’ ophthalmopathy. EyeM: Eye muscle membrane.

Figure 4: Anti-eye muscle cytosol (EyeC) IgM antibodies were associated with decreased eye muscle DIO2 activities in hyperthyroid Graves’ ophthalmopathy compared to those who did not have ophthalmopathy. EyeC: Eye muscle cytosol.

Discussion

In hyperthyroid Graves’ disease several autoantibodies are present with distinct binding reactivities. The pathognomonic role of anti-eye muscle antibodies in the development of Graves’ ophthalmopathy has not been clarified yet, although their perimysial or endomysial bindings to myofibers or connective tissue, such as fibroblast or adipose tissue were demonstrated. The bindings of anti-eye muscle antibodies did not result in any damagecellarly or intracellularly in eye muscle tissue. Therefore, the question comes always up whether these autoantibodies have any pathognomonic roles in the development of ophthalmopathy or they have only diagnostic roles. The fact, that sera of hyperthyroid Graves’ ophthalmopathy have a stimulating effect on orbital fibroblasts driving them to express chemoattractants, IGF-1 and stimulate hyaluronan synthesis [17-19].

Our findings highlighted that the role of DIO2 enzyme can play also as a target between orbital and thyroid diseases in hyperthyroidism, which is mainly present in the onset of Graves’ ophthalmopathy. Both, orbital and thyroid tissues contain DIO2 enzyme, which is essential for the maintenance of intracellular T3 pool. Besides DIO2, extraocular eye muscles contain also DIO3, while thyroid tissue DIO1 enzymes.

Deiodinases, DIO2 and DIO3 are involved in myogenesis, therefore in skeletal muscle like eye muscle physiology influencing the myofiber type composition and their capacity for regeneration [20].

In this study, the association of anti-eye muscle membrane and cytosol isotype antibodies with eye muscle DIO2 activities was investigated in hyperthyroidism. Our findings confirmed that EyeM-IgG and EyeC-IgG antibodies were associated with increased eye muscle DIO2 activities, while EyeM-IgM antibodies decreased them. The decreased eye muscle DIO2 activities were connected to smaller and the increased DIO2 activities to greater eye muscle thicknesses highlighting the role of DIO2 in eye muscle enlargements in hyperthyroid Graves’ ophthalmopathy. In thyroid autoimmunity, the occurrence of antibodies to DIO2 enzyme was demonstrated in a linkage with anti-pituitary antibodies [21].

In conclusion, anti-eye muscle isotype antibodies were associated with increased or decreased eye muscle DIO2 activities affecting eye muscle thicknesses in hyperthyroid Graves’ ophthalmopathy. Our results confirmed that DIO2 enzyme can be a new target between orbital and thyroid diseases in hyperthyroid Graves’ ophthalmopathy.

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References

1. Bahn RS (2010) Graves’ ophthalmopathy. N Engl J Med 362: 726-738.
2. Gunji K, De Bellis A, Kubota S, Swanson J, Wengrowicz S, et al. (1999) Serum antibodies against the flavoprotein subunit of succinate dehydrogenase are sensitive markers of eye muscle autoimmunity in patients with Graves' hyperthyroidism. J Clin Endocrinol Metab 84: 1255-1262.
3. Gopinath B, Musselman R, Beard N, El-Kaissi S, Tani J, et al. (2006) Antibodies targeting the calcium binding skeletal muscle protein calsequestrin are specific markers of ophthalmopathy and sensitive indicators of ocular myopathy in patients with Graves’ disease. Clin Exp Immunol 145: 56-62.
4. Smith TJ, Tsai CC, Shih MJ, Tsai S, Chen B, et al. (2008) Unique attributes of orbital fibroblasts and global alterations in IGF-1 receptor signaling could explain thyroid-associated ophthalmopathy. Thyroid 2008; 18: 983-988.
5. Molnár I, Kaczur V, Boros A, Krajczár G, Baláz C (1995) IgA autoantibodies against human eye muscle antigen detected by western blotting and immunohistochemical methods in Graves’ disease. J Endocrinol Invest 18: 408-414.
6. Molnár I, Balázs C (1994) IgA anti-eye muscle cytosol autoantibodies and the clinical stage in Graves’ disease. J Endocrinol Invest 17: 671.

7. El-Kaissi S, Wall JR (2012) Determinants of extraocular muscle volume in patients with Graves’ disease. J Thyroid Res 2012: 368536.

8. Wiersinga WM, Regensburg NI, Mourits MP (2013) Differential involvement of orbital fat and extraocular muscles in Graves’ ophthalmopathy. Eur Thyroid J 2: 14-21.

9. Salvatore D, Simonides WS, Dentice M, Zavacki AM, Larsen PR (2014) Thyroid hormones and skeletal muscle--new insights and potential implications. Nat Rev Endocrinol 10: 206-214.

10. Wang YY, Morimoto S, Du CK, Lu QW, Zhan DY, et al. (2010) Up-regulation of type 2 iodothyronine deiodinase in dilated cardiomyopathy. Cardiovasc Res 87: 636-646.

11. Bianco AC, Kim BW (2006) Deiodinases: implications of the local control of thyroid hormone action. J Clin Invest 116: 2571-2579.

12. Baqui MM, Gereben B, Harney JW, Larsen PR, Bianco AC (2000) Distinct subcellular localization of transiently expressed types 1 and 2 iodothyronine deiodinases as determined by immunofluorescence confocal microscopy. Endocrinol 141: 4309-4312.

13. Barrio-Barrio J, Sabater AL, Bonet-Farriol E, Velázquez-Villoria Á, Galofre JC (2015) Graves’ ophthalmopathy: VISA versus EUGOGO classification, assessment, and management. J Ophthalmol 249125: 1-16.

14. Molnár I, Balázs C (1991) Comparative study on IgG and IgA antibodies against human thyroid and eye-muscle antigens in Graves’ ophthalmopathy. Acta Med Hung 48: 13-21.

15. Molnár I, Czirják I (2000) Euthyroid sick syndrome and inhibitory effect of sera on the activity of thyroid 5’-deiodinase in systemic sclerosis. Clin Exp Rheumatol 18: 719-724.

16. Kaczur V, Vereb G, Molnár I, Krajczár G, Balázs C (1995) Kinetic characterization of thyroid peroxidase enzyme and its inhibition by autoantibodies. Acta Microbiol Immunol Hung 42: 345-350.

17. Pritchard J, Horst N, Cruikshank W, Smith TJ (2002) Igs from patients with Graves’ disease induce the expression of T cell chemoattractants in their fibroblasts. J Immunol 168: 942-950.

18. Smith TJ, Hoa N (2004) Immunoglobulins from patients with Graves’ disease induce hyaluronan synthesis in their orbital fibroblasts through the self-antigen, insulin-like growth factor-I receptor. J Clin Endocrinol Metab 89: 5076-5080.

19. Krieger CC, Neumann S, Place RF, Marcus-Samuels B, Gershengorn MC (2014) Bidirectional TSH and IGF-1 receptor cross-talk mediates stimulation of hyaluronan secretion by Graves disease immunoglobulins. J Clin Endocrinol Metab 100: 1071-1077.

20. Dentice M, Marsili A, Zavacki A, Larsen PR, Salvatore D (2013) The deiodinases and the control of intracellular thyroid hormone signaling during cellular differentiation. Biochim Biophys Acta 1830: 3937-3945.

21. Nakahara R, Tsunekawa K, Yabe S, Nara M, Seki K, et al. (2005) Association of antipituitary antibody and type 2 iodothyronine deiodinase antibody in patients with autoimmune thyroid disease. Endocr J 52: 691-699.