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Research Article

Non-Thyroidal Immunological Abnormalities in Patients with Euthyroid Graves' disease And Their Relatives

Hooshang Lahooti[1], Bernard[2], and Jack R Wall[1,2]*

[1]*Department of Medicine, University of Sydney, Nepean Clinical School, Australia*
[2]*Department of Medicine Notre Dame University of Australia, Sydney, NSW, Australia*

***Corresponding author**
Jack R Wall, Department of Medicine, University of Sydney, Australia, Telephone; 0402890919

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Abstract

Background: The ophthalmopathy associated with Graves's hyperthyroidism, called Graves's ophthalmopathy, is an autoimmune disorder of the extra-ocular muscles and orbital connective tissue. In about 10% of patient with ophthalmopathy thyroid function tests are normal and serum thyroid antibodies are not detected. This is called "Euthyroid Graves' Disease". Because autoantibodies targeting the skeletal muscle protein Calsequestrin and type XIII Collagen are detected in both Graves's ophthalmopathy and Euthyroid Graves' Disease it might be assumed that Euthyroid Graves' Disease is also autoimmune, although this has not been proven.

Clinical Subjects and Methods: In order to test the hypothesis that Euthyroid Graves' Disease is an autoimmune disorder, we first carried out a database search of EMBO, Google, Google scholar and PubMed for studies that looked for a personal or family history of autoimmunity, autoimmune markers such as vitiligo and alopecia or serum antibodies against autoantigens associated with various organ specific and multisystem autoimmune disorders Secondly, we studied 13 of our own patients with Euthyroid Graves' Disease, including a patient with complex Euthyroid Graves' Disease manifest as orbital swelling and high serum levels of Collagen XIII antibodies, for these autoimmune abnormalities We also tested their sera for Calsequestrin and type XIII collagen antibodies.

Results: Data base searches showed that there have been no studies addressing the existence of non-thyroidal Immunological abnormalities, markers or autoantibodies, and their prevalences in patients with Euthyroid Graves' Disease and their family members. In a retrospective studied of 13 of our own patients with Euthyroid Graves Diseases we found that while the prevalence of other immunological abnormalities in patients with Euthyroid Graves' Disease was similar to than in a control group of age, but not sex matched patients with Graves Ophthalmopathy, the prevalence of immunological abnormalities in their relatives was significantly less than in relatives of patients with Graves ophthalmopathy.

Discussion: While the findings in this preliminary study suggest that Euthyroid Graves' Disease may not be an autoimmune disorder, the results are fairly weak and inconsistent with findings in an earlier study in which we reported a high prevalence of cytotoxic antibodies against fresh thyroid follicular cells and significant bands on Western blotting in many of the same patients with Euthyroid Graves' Disease. Based on the two studies we propose that in Graves' ophthalmopathy the primary immune reaction is in the thyroid gland land whereas in Euthyroid Graves' Disease. The first reaction may be in the orbital tissue, the association between ophthalmopathy and thyroid autoimmunity being due to immunological cross reactivity in both disorders.

Conclusions: To further address the nature of, and relationship between, the orbital and thyroid reactions in Euthyroid Graves' Disease a larger prospective study of patients in whom B and T cell tests for immune reactivity against orbital and thyroid antigens, including the new candidate antigen IgF-1 Receptor, needs to carried out. New clinical tests for differences between Graves's ophthalmopathy and Euthyroid Graves' Disease and qPCR methods to identify new thyroid and orbital shared autoantigens may provide new information about the two types of what can be called "auto-immune ophthalmopathy and its management".

INTRODUCTION

The ophthalmopathy associated with Graves' hyperthyroidism is sometimes present without any apparent thyroid dysfunction or thyroid immunological abnormalities, where it is called "Euthyroid Graves' disease (EGD) or "euthyroid ophthalmopathy" [1-3].

The literature regarding the pathogenesis of EGD is confusing and controversial for many reasons, in particular because some authors consider that the detection of TSH receptor (TSH-R) antibodies is consistent with the definition of "Euthyroid Graves' Disease" even with normal thyroid function [4,5]. Others [6.7] have postulated that patients with EGD actually have Graves' ophthalmopathy (GO) where TSH-R antibodies were presumed to have been present in the past before the development of ophthalmopathy, or will be detected in the future.

Because GO is generally accepted to be an autoimmune disorder it might be assumed that EGD is also autoimmune. Indeed, the antibodies against Calsequestrin (CSQ) sand type XIII Collagen (coll XIII) that are found in GO [8-11] are also detected in patients with EGD (Lahooti, Wall et al unpublished observations)., GO is characterized by antibodies against the

IgF1-receptor (IgF1-R), possibly co-expressed with the TSH-R [12,13], However, according to the generally held definition of EGD, as discussed above TSH R antibodies are not detected in patients with EGD and IgF1-R antibodies have not yet been tested for. As described in the classical publication by Milgram and Wilensky [14], the characteristics of an autoimmune disease comprise I) the identification of specific autoantigens in the target tissue/organ ii) the corresponding serum autoantibodies iii) personal history of other immunological abnormalities iv) histological evidence of lymphocyte infiltration in the target tissue (es) v) family history of the same or other autoimmunity and vi) the ability to produce an animal model for the disease. We are not aware of any studies that directly test the hypothesis that EGD is an autoimmune disorder

In this preliminary study, we have studied 13 patients with EGD and a control group of 16 patients with GO, and their available relatives, for clinical evidence of non-thyroid autoimmunity and their sera for non-thyroid autoantibodies. We show that while the prevalence of autoimmune disorders and markers are similar in EGD and GO patients, their prevalences in relatives of patients with GO is significantly greater than in relatives of patients with GO. Before that we carried out a data base search for similar studies carried out in the past by others. There were none so we were encouraged to perform the above clinical study to test our hypothesis.

CLINICAL SUBJECTS AND METHODS

We examined patients with EGD and Graves ophthalmopathy (GO) and their relatives for autoimmunity and autoimmune markers and tested their sera for various non-thyroidal auto-antibodies to test the hypothesis that EGD, unlike GO, is not an autoimmune disorder. We studied 13 patients, including the above special patients, 5 males and 8 females aged 40 – 70 (mean age 66 yr.) with EGD and, as controls, 16 patients 5 males and 11 females aged 18 – 75 (mean- age 48 yr.) with GO who were selected at random from representative patients seen at the University of Sydney Thyroid clinics during the study period. The presence of ophthalmopathy was defined as a NOSPECS class [15] of 2 or more and a CAS score [16] of 3 or more, i.e., all patients had active disease with periorbital swelling epiphora, chemosis and conjunctival injection, and a few had eye muscle involvement as well. The severity of the clinical features and treatment modalities of the patients in the two ophthalmopathy groups were similar. All patients were seen by the same single observer (JW) each 6 or 12 weeks.

Serum orbital and other antibody tests were carried out in the context of the patients' clinical management. The diagnoses of other autoimmune disorders including rheumatoid arthritis, celiac disease and type 1 diabetes were according to routine clinical criteria. In patients and their relatives. Where possible, family histories were confirmed from patients' descriptions of the symptoms and tests carried out but, in all cases, we assumed that the diagnoses were accurate (we took the patients word for it)

Inclusion criteria for both groups were; age 16 – 80, a definite diagnosis of EGD and GO based on the clinical features and immunological tests and where possible long-term follow up. Exclusion criteria were age < 16 or > 80or complicating co-morbidities that made clinical follow up visits difficult or blood testing problematic to interpret. The study was carried out at the University of Sydney Thyroid Clinics. The Nepean Mountains Human Ethics Committee approved the study. In 2014. Because the study was retrospective and anonymous consent forms were not needed. The data were stored for 7 years then destroyed.

METHODS

Enzyme-linked immunosorbent assay

The Enzyme-linked immunosorbent assay (ELISA) for measurement of CSQ and coll XIII antibodies. The ELISA method has been described in previous publications from this laboratory [9,11] Tests were performed in triplicate, in 96 well plates. The optimal concentrations of purified calsequestrin and Coll XIII were found, in preliminary assays; to be one pg/ml for each protein and optimal serum dilution was 1/50 for CSQ and 1/25 for coll XIII. The second antibody was an alkaline phosphatase• labelled goat anti-human IgG diluted 1/4000. Results were expressed as optical density (OD) at 410 NM and a positive test taken as an OD > mean + 2SD for a panel of age and sex-matched healthy male subjects. The rationale for using only males in the reference group is discussed in previous publications in our laboratory (refs, 8-11) Other antibody tests were carried out by local Pathology laboratories with a 3 day turn around and CSQ and cool XIII ELISA tests were performed in the authors laboratory each week, The data were analyzed together at the end of the study, in 2020.

Statistical Analyses

Mean prevalence of immunological markers and abnormalities in patients with EGD and GO were compared using one-way ANOVA statistical analyses and Pearson's correlation coefficient and Spearman's P-value. For all analyses, a P value of < 0.05 was considered to be significant. The Graph Pad Prism 8 statistical package was used for the analyses.

RESULTS

Data base search for studies of non-thyroid immunological abnormalities in patients with Euthyroid Graves' disease and their relatives

Before carrying out the clinical studies, we carried out a literature search of the internet data bases EMBO, Google, Google scholar and PubMed for studies that looked for evidence of a personal or family history of autoimmunity, autoimmune markers such as vitiligo, premature grey hair and alopecia and serum autoantibodies against a panel of autoantigens associated with various organ specific and multisystem autoimmune disorders in patients with EGD. Computer data base searches did not identify any publications that addressed a possible

autoimmune pathogenesis for EGD by looking for presence of other immunological disorders, markers of autoimmunity such as alopecia or vitiligo or serum autoantibodies in patients with EGD. The key words used were; "Euthyroid Graves' disease, euthyroid orbitopathy or euthyroid ophthalmopathy" plus "autoimmunity, pernicious anemia, Addison disease, lupus. Rheumatoid arthritis, celiac disease, lupus, myasthenia Gravis, polymyositis, vitiligo, premature grey hair, alopecia" or; serum autoantibodies against; GPC, IF, nuclear antigens, IgF-1-R, smooth muscle antigen, mitochondrial antigen ("M") and Ig class gliadin antibodies or serum Vitamin B12 (for pernicious anemia). In other words, there been no previous studies addressing the pathogenesis of EGD by focusing on possible links with other immunological disorders, markers or autoantibodies

Detailed study of an interesting and seminal patient with congestive variant of EGD

Next, we studied a challenging patient with unusual EGD, a 50-year-old Asian woman who developed marked congestive ophthalmopathy in 2003, 5 years before she was first seen at the University of Sydney Thyroid Clinic, Nepean Hospital. She is included as a representative patient with personal the study period her eyes had been variably itchy, gritty, watery and swollen (CAS range 4-10) with intermittent flare ups requiring treatment with oral prednisolone (50 mg/day reducing over a few weeks) or steroid eye drops, or both. She had never complained of double vision or reduced vision and eye muscle function had always been normal. She was a nonsmoker. CT scan of her orbits carried out a year apart, in 2005 and 2006, showed normal eye muscle volumes. She had no upper eyelid lag or retraction or proptosis at any stage. The patient did not have associated thyroid autoimmunity. Specifically, she had no goiter or symptoms of thyroid dysfunction and serum levels of TSH and fT4 were normal throughout. Thyroid ultrasound carried out on several occasions over the 5 yr. by the senior author revealed a normal looking thyroid with normal echogenicity throughout and a single small nodule. When seen at the Thyroid clinic in October 2008, eye exam again revealed marked bilateral conjunctival injection, chemosis and peri orbital swelling, and her eyes were very watery (CAS 10). Her eyelids were swollen but she had no upper eyelid lag or retraction (Upper eyelid retraction (UER) score 0). Eye movements in all gazes were normal and she had no diplopia. She had no exophthalmos (13mm Jpn both eyes). She remains euthyroid with no evidence for thyroid disease and her congestive ophthalmopathy, although severe, is now stable. Current therapy includes steroid eye drops for eye itchiness and irritation and avoidance of bright light, smoke and dust.

Serum orbital (CSQ, coll XIII) antibody levels, major eye signs and any treatments, are summarized in Table 1. Serum antibodies against CSQ and coll XIII were measured on 6 occasions. While CSQ antibodies were negative in all samples, coll XIII antibodies were positive in 5 out of the 6 samples available, strongly so initially, mildly positive in the subsequent 2 tests, then negative, then positive again in the last two tests carried out, in each case corresponding generally to activity of her congestive ophthalmopathy (Table 1).

Table 1: Eye findings, thyroid function and immunological abnormalities in a patient with Euthyroid Graves' Disease manifested as isolated congestive ophthalmopathy.

Visit no.	Treatment	[1] Ophthalmopathy					[2] Orbital antibodies	
		Nunery	NOSPECS	CAS	UER score	Activity	CASQ1	Coll XIII
1	Nil	1	2	8	0	Yes	161	492
2	Nil	1	2	8	0	No	105	203
3	Nil	1	2	8	0	Yes	38	280
4	Nil	1	2	8	0	No	55	106
5	Steroid drops	1	2	10	0	Yes	121	200
6	Steroid drops	1	2	10	0	Yes	153	207

[1]Nunery = without (1) or with (2) eye muscle involvement. NOSPECS classes [15], CAS

Vivity Score [1-10,16[1,3]Measured in standard enzyme-linked immunosorbent (ELISA). CSQ = skeletal muscle calsequestrin, Coll XIII = type XIII collagen. A positive test was

Taken as an OD > the upper limit of normal for 30 healthy males namely, 194 for calsequestrin anti

Bodies and 175 for collagen XIII, antibodies

TSH-R antibodies were tested on stored sera in a single assay at the end of the study while all other tests were performed after each clinic visit. Thyro-peroxidase (TPO), thyroglobulin (Tg) and TSHr antibodies were always negative. Anti-nuclear antibodies, double stranded (DS) DNA antibodies and antibodies against extractable nuclear antigens (ENA), tested on one occasion only, were also negative (results not shown).

Study of patients with Euthyroid Graves' Disease

Finally, we studied 13 of our own patients with EGD, including the patient with the congestive varian described in detail above. And, as controls, 16 age and sex matched patients with GO, for these abnormalities and tested their sera for eye muscle and orbital connective tissue antibodies in ELISA. These results are summarized in Table 1 as total "events" rather than number of patients or subjects since some patients had 2 or more events. We compared total events (other autoimmunity, non-thyroid antibodies, FH of these) in the two groups. The number of immunological abnormalities in patients with EGD (n=11) was similar to that in sex, but nit age, matched patients with GO (n=16) and the difference was not significant (T test, P=NS). On the other hand, the prevalence of abnormalities in their relatives (n=7) was much lower than in relatives of patients with GO (n=18) the difference being significant (t test, $p<0.05$).

Although we did not study normal contolrs, historical indications are that the prevaences of autoantidodeies is low, but not zero, in normal subjects, e'g' low tires of thyroid antibodies are found in about 5% of these subjects on data base search and own our past studies.. However autounnity is rare in normals (Table 2).

Table 2: Prevalence of autoimmune disorders, autoimmune markers and serum auto antibodies in patients with Euthyroid Graves' disease, Graves's ophthalmopathy and their relatives

Group (No. of patients)	Personal History				Total events (A) [3]	Family History		Total events (B)	Total Events A + B
	AITD [1]	Markers [2]	Autoantibodies			AITD	Markers		
			Orbital[4]	Other[5]					
EGD [6] (n-13)	3	2	6	0	11	7	0	18	29
GO [7] (n=16)	2	1	4	1	8	16	2	26	34
P value [8]	NS	NS	NS	NS	NS	P<0.05	NS	P<0.05	NS

[1]AITD = autoimmune thyroid disease; [2]Alopecia, prematurely grey hair, vitiligo; [3]Some patients had 2 or more features; [4]type XIII Collagen, Calsequestrin; [2]ANA, ENAs, GPC, IF, M, smooth muscle antigen (sMA), GAD, Acetyl Choline Receptor; [6]EGD = Euthyroid Graves' disease; [7]GO = Graves ophthalmopathy; [8]Determined using the student T test [9]NS – Not significant

DISCUSSION

EGD is a complex and somewhat controversial disorder. Firstly, there is no consistency in its naming; Ophthalmologists prefer to call the eye disorder" euthyroid orbitopathy" While most endocrinologists refer to the disease as EGD, a few prefer the inappropriate "Graves' ophthalmopathy with negative TSH R antibodies" This is the case because these patients do not have Graves' disease nor any other thyroid disorder on long term follow up [17]. Others have named the disease thyroid-associated ophthalmopathy of euthyroid patients [18], which is clearly wrong since they don't have Graves's ophthalmopathy. Although it seems appropriate to call the disease "Euthyroid Graves' disease" the best name may be "Autoimmune ophthalmopathy not associated with Graves' disease" to indicate that the eye disorder is the same as that in GO, although usually milder, and separate from Graves' disease, but that is too long to be popular.

Secondly, there is confusion about how we in interpret studies about EGD as there are different assays for TSH-R antibodies and some workers have claimed that those measured in the new and sensitive Thyretain bioassay, are not only more sensitive but specific to the ophthalmopathy of Graves' disease [19]. However, some workers also claim to be able to detect these antibodies in the majority of patients with EGD [20] whereas in our study we did not detect the antibodies in any patient with EGD while tests were positive in the great majority of patients with GO [21].

Thirdly, do patients with EGD have or develop, hyperthyroidism in the long term? Because some of our patients have been followed for up to 20 yr. [18] it seems highly unlikely that they have ever had the TSHR antibodies which are detected in patients with Graves' hyperthyroidism and postulated to cross react with the TSHR protein in the orbital connective tissue. So, one can conclude that while EGD is similar to GO, hyperthyroidism is not a component.

To summarize the main findings, we found no previous studies looking for family or personal history of other immunological abnormalities or markers in EGD, and in a prospective studied of 13 of our own patients with EGD we found that while the prevalence of other immunological abnormalities in patients with EGD was similar to than in a control group of age and sex-matched patients with GO, the prevalence of immunological abnormalities in their relatives was much less.

In an earlier study [22] patients with endocrine ophthalmopathy not associated with goitre, antithyroid microsomal or antithyroglobulin antibodies, or overt thyroid disease (i.e. EGD as defined)were investigated for evidence of cytotoxic antibodies in 22 patients We measured antibody-dependent cell-mediated cytotoxicity (ADCC) against fresh thyroid cells using a 51chromium release assay and, thyroid membrane-reactive antibodies in an ELISA incorporating solubilized thyroid membranes, and TSH receptor-binding antibodies using a radio receptor assay. We also carried out sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) and Western blotting for antibodies reactive with 64 and 110 kDa (thyroid peroxidase) membrane proteins. Bands were demonstrated on SDS-PAGE, at 64 and 110 kDa in 13 patients. ADCC tests were positive in 7 patients, and ELISA was positive in 4 of the 17 patients tested. In addition, TSH -R antibody tests were positive in 5 patients, none of whom had other evidence for hyperthyroidism. Finally, significant lymphocyte infiltration was demonstrated on aspiration biopsy in 3 patients. All 18 patients had positive tests in at least one of the immunological assays [22].

One way to reconcile the apparently conflicting results of fts two studies carried out by the sane authors on many of the same patients but using different thyroid immunological tests, is to develop a new hypothesis for the difference between EGD and GO namely; that in GO the primary reaction may be in the thyroid, with secondary cross reaction in the orbit, whereas in EGD the orbital reaction is primary and the thyroid reaction is secondary and less well developed, (hence the lower prevalence of family history of autoimmune markers). This might also explain results from other studies cited here in which TSH-R antibodies may or may not be detected. In order to further address the nature of, the orbital and thyroid reactions in EGD and their relationship, a large prospective study of patients in whom a large number of B and T cell tests for immune reactivity against orbital and thyroid antigens, including the current putative target antigen, the IgF-1-R, should be carried out

In this preliminary study we have compared groups of patients with EGD and GO in respect to the prevalence of non-thyroidal, autoimmune disorders or markers (alopecia vitiligo premature grey hair etc.) and serum autoantibodies While the prevalence of autoimmunity was similar in the two groups, relatives of patients with GO had a much greater prevalence of non-thyroid autoimmunity than in those of patients with EGD, suggesting that clinical and laboratory evidence for autoimmunity in EGD is less than that in the much more common GO.

Limitations of the study

Where I) the numbers studied were small due to availability of age matched patients at the Thyroid Clinics ii) we did not measure IgF1-R antibodies in the patients iii) although patients were followed for up to 20 years the study was not prospective as the data were only analyzed at the end of the study

CONCLUSIONS

While this is a preliminary finding, and the overall evidence favoring our hypothesis, can be described as "weak, the results do suggest that EGD may not be an autoimmune disorder. To further address the nature of, and relationship between, the orbital and thyroid reactions in Euthyroid Graves' Disease and Graves Ophthalmopathy a larger prospective study of age and sex matched patients (with EGD and GO) in whom B and T cell tests for immune reactivity against orbital and thyroid antigens, including the new candidate antigen, the IgF-1 Receptor. Needs to be carried out. We also need to measure other serum autoantibodies in the hope of identifying new abnormalities that might indicate alternative diagnosis. New clinical tests for Euthyroid Graves' Disease and qPCR methods to identify new thyroid and orbital shared autoantigens in the orbit may provide new information about the two different forms of what we may call "auto-immune ophthalmopathy" and new therapies

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REFERENCES

1. Burch HB, Wartofsky L. Graves' ophthalmopathy: Current concepts regarding pathogenesis and management. Endocr Rev. 1993; 14:747-793.

2. Tabasum A, Khan I, Taylor P, Das G, Okosieme OE. Thyroid antibody negative euthyroid Graves' ophthalmopathy. Endocrinol Diabetes Metab Case Rep. 2016; 2016: 160008.

3. Moore EA, Moore LM. Advances in Graves' disease and Other Hyperthyroid Disorders. Book chapter, McFarland Health Series ISBN. 2013; 78: 189-198.

4. Kasagi K, Hatabu H, Tokuda Y, Iida Y, Endo K, et al. Studies on thyrotrophin receptor antibodies in patients with euthyroid Graves' disease Clin Endocrinol (Oxf). 1988; 29:357-368.

5. Watanabe M, Iwatani Y, Kashiwai T, Iijima T, Fujikado T. Euthyroid Graves' disease showing no thyroid abnormalities except positive thyroid-stimulating antibody (TSAb): two case reports. J Intern Med. 1995; 238: 379-384.

6. Kosugi S, Inoue D, Sugawa H, Enomoto T, Mori T. Similarity and dissimilarity between clinical and laboratory findings, especially anti-thyrotropin receptor antibody in ophthalmic Graves' disease without persistent hyperthyroidism and hyperthyroid Graves' disease. Endocrinol Jpn. 1990; 37: 343-354.

7. Kashiwai T, Tada H, Asahi K, Hidaka Y, Tamaki H, et al. Significance of thyroid stimulating antibody and long term follow up in patients with euthyroid Graves' disease. Endocr J. 1995; 4: 405-412.

8. Gunji K, Kubota S, Stolarski C, Wengrowicz S, Kennerdell J, et al. A 63 kDa skeletal muscle protein associated with eye muscle inflammation in Graves' disease is identified as the calcium-binding protein calsequestrin. Autoimmunity. 1999; 29:1-9.

9. Gopinath B, Wescombe, Nguyen B, Wall JR. Can Autoimmunity against calsequestrin explain the eye and eyelid muscle inflammation of thyroid eye disease? Orbit. 2009; 28: 256-261.

10. Salvi M, Fukazawa H, Hiromatsu Y, Triller H, Bernard N, et al. Role of autoantibodies in the pathogenesis of endocrine autoimmune disorders and in their association. Endocr Rev. 1988; 9: 450-466.

11. Gopinath B, Musselman R, Adams C, Tani J, Beard N. Study of serum antibodies against three eye muscle antigens and the connective tissue antigen collagen XIII in patients with Graves' disease with and without ophthalmopathy – correlation with clinical features. Thyroid. 2006; 16: 967-974.

12. Krieger CC, Perry JD, Morgan SJ, Kahaly GJ, Gershengorn MC, TSH/IGF-1 Receptor Cross-Talk Rapidly Activates Extracellular Signal-Regulated Kinases in Multiple Cell Types. Endocrinology. 2017; 158: 3676-3683.

13. Smith TJ, Jansen JAMJL. Insulin-like Growth Factor-I Receptor and Thyroid-Associated Ophthalmopathy. Endocr Rev. 2019; 40: 236-267.

14. Milgrom F, Witebsky F. Autoantibodies and autoimmune diseases. JAMA. 1962; 181: 706-716.

15. Werner SC. Classification of the eye changes of Graves' disease. J Clin Endocrinol Metab. 1969; 29: 982-984.

16. Mourits MP, Prummel MF, Wiersinga WM, Koornneef L. Clinical activity score as a guide in the management of patients with Graves' ophthalmopathy. Clin Endocrinol (Oxf). 1997; 47: 9-14.

17. McCorquodale T, Lahooti H, Gopinath B, Wall JR. Long term follow-up of seven patients with ophthalmopathy not associated with thyroid autoimmunity: heterogeneity of autoimmune ophthalmopathy. Clin Ophthalmol. 2012; 1063: 1063-1071.

18. Suzuki N, Noh JY, Kameda T, Yoshihara A, Ohye H, Suzuki M, et al. Clinical course of thyroid function and thyroid-associated ophthalmopathy in patients with euthyroid Graves' disease. Clin Ophthalmol. 2018; 12:739-746.

19. Lytton SD, Ponto KA, Kamitz M, Koln LD, Kahaly GJ, Matheis N. Novel thyroid stimulating immunoglobulin bioassay is a functional indicator of activity and severity of Graves's orbitopathy. J Clin Endocrinol Metab. 2010; 95: 2123-2131.

20. Lytton SD, Kahaly GJ. Bioassays for TSH-Receptor autoantibodies: an update. Autoimmun Rev. 2010; 10: 110-122.

21. Lahooti H, Tran HA, El Kochairi I, Lytton SD, Champion BC, Wall JR, et al. TSH receptor antibodies as measured in a thyroid stimulating immunoglobulin (TSI) reporter bioassay are not detected in patients with euthyroid Graves' disease. Austin J Clin Ophthalmol. 2014; 1: 1024.

22. Salvi M, Zhang ZG, Haegert D, Woo M, Liberman A, Cadarso L, et al. Patients with endocrine ophthalmopathy not associated with overt thyroid disease have multiple thyroid immunological abnormalities. J Clin Endocrinol Metab. 1990; 70: 89-94.