Serum Thyroglobulin Levels in Patients with Thyroid Autoimmunity with and without Ophthalmopathy or Isolated Upper Eyelid Retraction (January 2015)

H. Lahooti, MD, T. Shanmuganathan MD¹, B. Champion, J.R. Wall, MD¹*

Abstract — Background: An old hypothesis for the pathogenesis of the ophthalmopathy associated with Graves hyperthyroidism is that thyroglobulin (TG) passes from the thyroid gland via lymphatics and blood vessels to the orbital tissues where it binds to the eye muscles and is targeted by circulating TG antibodies, which leads to eye muscle and orbital connective tissue inflammation. Serum TG may be a marker for this reaction. Methods: We measured serum TG concentrations in patients with Graves disease, Hashimoto thyroiditis, transient (silent, subacute) thyroiditis, with and without ophthalmopathy or isolated upper eyelid retraction, control patients with thyroid nodules, and normals, correlating with parameters for the activity and severity of any ophthalmopathy or upper eyelid retraction (UER) and serum levels of TSH receptor (TSHR) antibodies measured as TRAb. Results: Fifty-two % of patients with GO had increased TG levels compared to 26% of those with no ophthalmopathy. Although the relationship was not close, serum TG levels generally correlated with ophthalmopathy in patients with Graves’s disease and most patients with severe and active eye disease had high levels of TG, usually associated with high titres of TSHR antibodies. In patients with Hashimoto thyroiditis and transient thyroiditis there were no significant correlations between TG levels and either ophthalmopathy or isolated UER. Conclusion: the study suggests that TG may play a role in the development of the ophthalmopathy associated with Graves disease, but not Hashimoto thyroiditis, and can be considered a marker for Graves-associated orbital autoimmunity. The results provide further evidence that the pathogenesis of the eye changes associated with Hashimoto thyroiditis is different from that in GO.

Keywords — Enter 3 to 7 keywords, separated by commas.

I. INTRODUCTION

The ophthalmopathy associated with Graves’ hyperthyroidism, Graves ophthalmopathy (GO), is most likely a complex T lymphocyte mediated disorder, although most investigators believe that autoantibodies directed against the TSH receptor (TSHR) expressed on the surface of the orbital fibroblasts, pre-adipocytes and fibrocytes are the main drivers of the orbital reactions [1-4]. However, we have recently found that TSHR antibodies, as measured in a novel chimeric cell-based reporter bioassay for thyroid stimulating immunoglobulins (TSI), the TSI-reporter assay, are not detected in patients with euthyroid Graves disease unless they had converted to Graves hyperthyroidism [5] or in patients with Hashimoto thyroiditis and eye signs [6]. In addition, generally mild ophthalmopathy is also found in some patients with transient sub-acute or silent thyroiditis [6] in whom TSHR antibodies are not generally detected, suggesting that other theories for the pathogenesis of the eye changes should be investigated.

One hypothesis for the pathogenesis of Graves ophthalmopathy (GO) was formulated by Konishi et al [8] and McDougall et al [9], the so called “Kriss Hypothesis”, in the early 1970s. They postulated that the initiating event in the pathogenesis of GO was the accumulation of thyroglobulin (TG) in the orbital tissues, which they demonstrated [8, 9], followed by an autoimmune reaction against TG bound to the extra ocular muscles. These workers speculated that the orbital inflammatory reaction and eye signs were more severe on the side (left or right) on which patients slept, reflecting greater trafficking of antibodies, TG and mononuclear cells on that side, which could explain “unilateral” ophthalmopathy. On the other hand, using a monoclonal antibody against thyroglobulin (A3), we failed to demonstrate TG in orbital tissues from normal subjects [10]. Moreover, others found no correlation between serum titres of anti-thyroglobulin antibodies and the presence or severity of the ophthalmopathy [11]. However, recent evidence obtained using a variety of experimental approaches [12-14], provides renewed support for the TG hypothesis and reminding us that the orbital reaction in GO begins in the thyroid gland in the context of a vigorous and complex series of inflammatory reactions involving large numbers of mononuclear cell types, cytokines and adhesion
molecules in the course of which TG and other thyroid antigens could be released into the circulation.

Serum TG levels are often increased in patients with subacute thyroiditis [15] and following radiiodine treatment of Graves hyperthyroidism [16] reflecting increased thyroid inflammation in these situations. While increased serum TG levels might be expected in some patients with Graves disease and Hashimoto thyroiditis, higher levels in patients with ophthalmopathy compared to those with no eye signs would suggest that the orbital inflammation was closely associated with the thyroid reaction. Here, we have measured serum concentrations of TG in patients with Graves disease, Hashimoto thyroiditis and transient (silent, subacute) thyroiditis with and without ophthalmopathy or isolated upper eyelid retraction correlating with parameters of any associated ophthalmopathy and levels of TSHR antibodies measured in a binding assay. We showed a correlation between TG levels and ophthalmopathy in patients with Graves disease but not transient or chronic thyroiditis.

**Table 1.**

<table>
<thead>
<tr>
<th>Group</th>
<th>Number</th>
<th>Increased serum TG1 (n, %)</th>
<th>P value, ophth2. Vs. no ophth or UER3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Graves hyperthyroidism</td>
<td>31</td>
<td>8 (26%)</td>
<td>0.0472</td>
</tr>
<tr>
<td>Graves ophthalmopathy</td>
<td>23</td>
<td>12 (52%)</td>
<td></td>
</tr>
<tr>
<td>Hashimoto thyroiditis</td>
<td>42</td>
<td>8 (19%)</td>
<td></td>
</tr>
<tr>
<td>Hashimoto thyroiditis and ophthalmopathy</td>
<td>12</td>
<td>3 (25%)</td>
<td>0.6926</td>
</tr>
<tr>
<td>Hashimoto thyroiditis and isolated UER</td>
<td>13</td>
<td>3 (22%)</td>
<td>0.7093</td>
</tr>
<tr>
<td>Euthyroid Graves disease</td>
<td>4</td>
<td>0 (0%)</td>
<td>NA4</td>
</tr>
<tr>
<td>Transient thyroiditis</td>
<td>7</td>
<td>2 (28%)</td>
<td>NA</td>
</tr>
<tr>
<td>Multi nodular goitre</td>
<td>24</td>
<td>12 (50%)</td>
<td>NA</td>
</tr>
<tr>
<td>Normals</td>
<td>10</td>
<td>0 (0%)</td>
<td>NA</td>
</tr>
</tbody>
</table>

1 TG = thyroglobulin
2 ophth = ophthalmopathy
3 UER = upper eyelid retraction
4 NA = not applicable

**II. MATERIALS AND METHODS**

**Patients**

Serum TG measurements were performed in patients with:

1) Graves disease; 4 males and 50 females of whom 23, one male and 22 females aged 21 - 64 (mean age 38 yr) had ophthalmopathy and 31, 3 males and 28 females aged 21 - 64 (mean age 40 yr), had no eye signs.

2) Hashimoto thyroiditis; 11 males and 55 females of whom 12, one male and 11 females aged 20 - 62 (mean age 52 yr) had ophthalmopathy, 13, one male and 12 females aged 21 – 62 (mean age 35 yr), had isolated upper eyelid retraction (UER) and 41, 9 males and 32 females aged 28 – 70 (mean age 63 yr) had no eye signs.

3) Euthyroid Graves disease, 2 males aged 63 and 41 and two females aged 75 and 64 (overall mean age 61 yr).

4) Transient (silent, subacute) thyroiditis, all females of whom one, aged 50 had ophthalmopathy, 2 aged 30 and 30, had isolated UER and 4, aged 27-70 (mean age 42 yr) had no eye signs.

5) Multi nodular goitre; 2 males and 23 females aged 16 – 69 (mean age 58 yr), none of whom had any eye signs.

6) Normal subjects; 2 males and 8 females aged 16 – 69 (mean age 48 yr) as controls.

The diagnosis of the various thyroid disorders was made according to the usual clinical features and confirmed from thyroid function testing, serum TPO and TG antibodies and real-time thyroid ultrasonography. The retrospective study was approved by the Nepean Hospital Human Ethics Committee. Consent forms were not required.

**Eye assessment**

Any associated ophthalmopathy and/or UER was assessed as; i) Nunery type 1 (without restrictive myopathy) or type 2 (with restrictive myopathy) [17] ii) a modified Clinical Activity Score (CAS) (0-12) of Mourits et al. [18] which is a measure of disease activity and includes a score of 1-3 for UER (not included in the original CAS) iii) Werner’s NOSPECS classes and scores (0-15) [19] 4) an UER score, determined from scores of 0-3 for each of upper eyelid retraction and upper eyelid lag, for each eye (0-12) and a total eye score, being the sum of the CAS, NOSPECS score and UER score (0-39) as a measure of the overall severity of the ophthalmopathy. The degree of proptosis (mm) was measured using a Hertel exophthalmometer where a positive reading was defined as > 18 mm in either eye or ≥ 2 mm difference between the eyes. For the purpose of the study “ophthalmopathy” was taken as a NOSPECS class ≥ 2, regardless of the CAS, which is a measure of activity but not severity and an UER score of ≥ 2 being taken as significant UER.

**Measurement of serum thyroglobulin**

Serum TG was measured by Barratt & Smith Pathology, Sydney, Australia, using standard commercial kits according to the manufacturers instructions. The normal range is 0-30 ugm/L.

**Other tests**

Measurements of plasma freeT4 (fT4), free T3 (fT3) and TSH and serum TPO and TG antibodies was carried out by Barratt & Smith Pathology, Sydney, Australia, according to the manufacturers’ instructions. Serum TSHR antibodies were measured in the TRAb assay (reference range < 2.1 IU/L) by Barratt & Smith Pathology, according to the manufacturers’ instructions.

**Statistical Analysis**
Statistical analysis was carried out using SigmaStat (version 2.0; Jandel Co., San Rafael, CA, USA). Mean (± SE) TG for groups of patients were compared using the Mann-Whitney non-parametric test. Prevalences of increased serum TG in the various groups were compared statistically using the X² test and correlations between serum TG levels and i) total ophthalmopathy scores ii) UER scores and iii) serum TSHR antibody titres were assessed using the Pearson r test. In all tests a p-value of < 0.05 was taken as significant.

III. RESULTS

We have measured serum TG levels in patients with Graves disease, transient (silent, subacute) and chronic (Hashimoto) thyroiditis and control patients and subjects, correlating with parameters of any associated eye changes or UER and serum titres of TSHR antibodies measured as TRAb. We excluded patients who had undergone thyroidectomy or radioactive iodine ablation in whom serum TG levels were expected to be very low or undetectable (< 0.9 ugm/L). The prevalences of increased serum TG (>30 ugm/L) in the various groups are shown in table 1. Fifty-two % of patients with GO had increased TG levels compared to 26% of those with no eye signs and the difference was significant (X² test, p 0.0472). On the other hand, in patients with Hashimoto thyroiditis and transient thyroiditis the differences between groups with and without ophthalmopathy or isolated UER were not significant for either group (X² test, p = NS). Compared to the normals, GO was the only group where mean (+/- SE) was significantly greater than that for the normal subjects, all of whom had no eye signs and TG levels < 30 ugm/L (fig. 1).

Next, we correlated serum TG levels with parameters of the ophthalmopathy in patients with GO (fig. 2) and Hashimoto thyroiditis with eye changes (fig. 3) or UER (fig. 4). The overall severity of the ophthalmopathy was measured as total eye score (CAS + NOSPECS score + UER score; 0-39) and the severity of any UER in patients with Hashimoto thyroiditis (where it may be the only sign) was measured as an UER score (0-12). There were no significant correlations between TG levels and total eye score (Pearson r = -0.01231, p = NS) in patients with GO (fig. 2) or total eye score (Person r = -0.01231, p = NS) (fig. 3) or UER score (Pearson r = -0.2876, p = NS) (fig. 4), in patients with Hashimoto thyroiditis. In patients with transient thyroiditis in whom eye disease was less frequent, less severe and often limited to isolated UER; there were no significant correlations in these groups between TG levels and either total eye score or UER score (results not shown).
Patients with high levels of TG tended to have ophthalmopathy and higher serum levels of TRAb; indeed, the overall correlations between serum TG and TRAb levels in the 17 patients with GO tested was highly significant (Pearson r = 0.9578, p < 0.0001) (fig. 5).

Fig.2. Correlation between serum thyroglobulin levels and ophthalmopathy severity, measured as total eye score (0 - 39), determined using the Pearson r test, in patients with Graves ophthalmopathy; r = -0.01231, p = 0.9578.

Fig.3. Correlation between serum thyroglobulin levels and ophthalmopathy severity, measured as total eye score (0 - 39), determined using the Pearson r test, in patients with Hashimoto thyroiditis; r = -0.9513, p < 0.0001) (fig. 5)

IV. DISCUSSION
Most researchers believe that Graves ophthalmopathy is initiated by antibodies targeting the TSHR expressed in the orbital preadipocytes, fibroblast and fibrocytes [1-4], probably in association with IGF-I [20, 21] and that eye muscle damage is secondary to this connective tissue inflammation. Proponents of this notion propose that other thyroid antigens namely, TG and thyroid peroxidase, are also expressed in the orbital tissues and targeted in the autoimmune reactions [22, 23]. Whilst this cannot explain the pathogenesis of ophthalmopathy in the absence of thyroid disease, so called “euthyroid Graves disease” or in the eye changes in 25% of patients with Hashimoto thyroiditis in whom TSHR antibodies are not usually detected [24, 25], it remains a valid hypothesis for the development of ophthalmopathy in patients with Graves disease.

Earlier studies by the Kriss and co-workers [8, 9] suggested that ophthalmopathy was caused by autoimmunity against TG which was bound in the orbital tissues, especially the extraocular muscles, following its homing from the thyroid tissue through the lymphatics and blood vessels in the neck. Although subsequent studies suggested that TG was not present in the orbit [10], this remains controversial and recent studies [11-13] have raised the possibility that TG may indeed be an important antigen targeted in the orbital reactions of GO.

Because we have measured serum TG in patients with various autoimmune thyroid disorders we were able to examine the correlation between serum TG levels and corresponding eye changes in patients with Graves disease, Hashimoto thyroiditis and transient thyroiditis. To summarise the main findings; although the relationship was not close, some patients with GO with severe eye disease had high serum levels of TG, usually associated with high titres of TSHR antibodies, and overall there was a significant correlation between TG levels and the presence of ophthalmopathy in patients with Graves disease. In patients with Hashimoto thyroiditis and transient thyroiditis, where eye changes are usually less severe and less frequent, there was no correlation between TG levels and parameters of the eye disease.

In our recent studies we showed that TSHR antibodies measured in the TSI bioassay Thyreporter bioassay were not detected in patients with euthyroid Graves disease [5] or Hashimoto thyroiditis [6]. Because TSI measured in a Thyreporter bioassay is a good marker for ophthalmopathy in patients with Graves disease [26, 27] the negative findings in our studies suggest that the eye changes associated with Hashimoto thyroiditis, which are usually milder than those in Graves disease and often restricted to upper eyelid retraction, cannot be explained by targeting the TSHR. The findings reported here provide further evidence that the mechanism for development of ophthalmopathy in patients with Graves hyperthyroidism may be different from that in HT.

Strengths and weaknesses of the study
The strength of our study is that part of the routine assessment of our patients with GH and HT is the measurement of serum TG. Because we recognize that isolated UER is common in patients with HT [28] we have developed a unique UER score, which allows us to quantify this important eye sign in both disorders, to supplement the well known CAS and NOSPECS scores. The main weakness of the study is that it was carried out retrospectively and thus we were unable to correlate TG levels with goitre size as a parameter of the severity of the thyroiditis. In order to further address a possible role of TG in the pathogenesis of ophthalmopathy, and its
relationship to known risk factors such as smoking status and serum levels of TSHR antibodies as measured in the new TSI reporter bioassay, a larger prospective study is planned. In this study we would also measure calsequestrin and collagen XIII antibodies, shown to be markers of the orbital reactions in our studies (29).

![Fig 4. Correlation between serum thyroglobulin levels and upper eyelid severity measured as an upper eyelid retraction (UER) score (0-12), determined using the Pearson r in patients with Hashimoto thyroiditis; test r = 0.9513, p = 0.2052.](image1)

![Fig 5. Correlation between serum thyroglobulin levels and serum TSHR antibody levels, measured as TRAb (normal < 2.1 IU/L), determined using the Pearson r test in patients with Graves ophthalmopathy; r = 0.9513, p = < 0.0001.](image2)

V. CONCLUSIONS

Although the results from our study support the hypothesis of Kriss and co-workers (8, 9) that TG from the thyroid gland may play a role in the development of ophthalmopathy in patients with Graves hyperthyroidism this has not been proven and more studies are needed. However, the results do provide further evidence that the pathogenesis of the eye changes associated with Hashimoto thyroiditis, where serum TG levels are usually normal, is different from that in the more common and severe ophthalmopathy found in 40% of patients with Graves hyperthyroidism.

ACKNOWLEDGMENTS

This research was supported by grants from Nepean Blue Mountains Local Health District and the Nepean Medical Research Foundation.

REFERENCES AND FOOTNOTES

Pathogenesis of Thyroid Eye Disease: important role of autoimmunity against calsequestrin and collagen XIII. A review.


Wall JR. The TSH-Receptor and Thyroid-Associated Ophthalmopathy—a Convenient Hypothesis with too many exceptions to be true. Int J Endocrinol Metab. 2007; 2: 49-51.


Dr Jack R Wall, MD PhD is Professor of Medicine at the University of Sydney and Director of the Thyroid Research Laboratory at Nepean Hospital, Kingswood NSW Australia. His research interests over many years have concerned the pathogenesis of thyroid autoimmunity, in particular the ophthalmopathy associated with Graves disease and Hashimoto thyroiditis. He and his colleagues have identified several orbital antigens which are targeted by antibodies and T lymphocytes.

Dr Thayalini Shanmuganathan BSc MPhil. PhD is a Research Fellow working with Professor Jack Wall’s thyroid team at the University of Sydney. She has completed her PhD at Lincoln University, New Zealand and Post-Doctoral Fellowship at CSIRO, Australia. Her study has been in the area of immune response and its impact on relevant gene expression levels. Currently, her research concerns the role of the thyroidal T lymphocytes in the pathogenesis of the ophthalmopathy associated with Graves’ disease and Hashimoto’s thyroiditis.

Bernard Champion B. Sc. (Med), M.B. B.S. (First Class Honours) UNSW in 1995. Master of Medical Education (M Med Ed) University of Sydney 2009. He is currently a Senior Lecturer and Head of Department Endocrinology at Sydney Medical School and Nepean Hospital. Since 2014 he has acted as Clinical Coordinator for the entire University of Sydney Medical Program. His research interest include thyroid disease, diabetes, clinical endocrinology and medical education with over 20 peer reviewed publication in these areas.

Dr Hoosgang Lahooti is a Molecular and Cellular Biologist who has studied various aspects of molecular endocrinology. He received his PhD in molecular and cellular biology from University of Bergen in Norway 1990. He is a Senior Scientist and Conjoint Lecturer with Sydney University Medical School, Nepean and is collaborating with Professor Jack Wall in studies of the pathogenesis of thyroid associated ophthalmopathy.