

Research paper

Identification of risk factors for the development of ophthalmopathy in patients with Hashimoto's thyroiditis

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23 ABSTRACT

24 **Background:** Thyroid related ophthalmopathy, or eye disease (TED), is more often
 25 associated with Graves' hyperthyroidism than Hashimoto's thyroiditis. In the latter disorder,
 26 the pathogenesis of the eye signs may be different and the influence of well-known risk
 27 factors for the development of eye signs in patients with Graves' hyperthyroidism, such as
 28 smoking, age and gender, have not been studied in detail. The aim of our study was to identify
 29 the risk factors which influence eye disease in patients with Hashimoto's thyroiditis.

30 **Methods:** A retrospective cross sectional study included 105 patients with Hashimoto's
 31 thyroiditis and investigated 6 potential risk factors namely age, gender, smoking, vitamin D
 32 deficiency and serum levels of antibodies to thyroid peroxidase (TPO) and thyroglobulin (Tg).
 33 A binary logistic regression was used to determine whether one or more of the factors were
 34 predictive for i) the development of ophthalmopathy ii) the development of upper eyelid
 35 retraction (UER) iii) the type of ophthalmopathy (congestive ophthalmopathy, ocular
 36 myopathy or both) or iv) the activity of the eye disease assessed as Clinical Activity Score
 37 (CAS), in patients with Hashimoto's thyroiditis.

38 **Results:** Our analyses showed a protective effect of ageing on the development of
 39 ophthalmopathy in patients with Hashimoto's thyroiditis, the risk decreasing by 5.4% for each
 40 additional year and a detrimental effect of smoking, with a risk of ophthalmopathy 5.5 times
 41 greater in smokers. High serum levels of TPO antibodies were found to be protective against
 42 the development of UER but not ophthalmopathy. None of the tested factors seemed to
 43 influence the risk of any ophthalmopathy subtype namely, congestive ophthalmopathy, ocular
 44 myopathy or mixed disease. However, gender has an effect on the activity of ophthalmopathy,
 45 men with Hashimoto's thyroiditis related eye disease being 18 times more likely to develop
 46 active ophthalmopathy than women.

47 **Conclusions:** Because the risk of ophthalmopathy in patients with Hashimoto's thyroiditis
 48 decreases with age older patients should be advised not to smoke as a preventive measure
 49 against ophthalmopathy development. Hashimoto's thyroiditis-related ophthalmopathy is
 50 more active in male patients who should therefore be monitored more closely. The
 51 mechanism for the observed risk reduction of UER development in the presence of high levels
 52 of TPO antibodies could be studied to help with our understanding of the pathophysiology of
 53 ophthalmopathy and the development of new therapies.

54

55 INTRODUCTION

56 The pathogenesis of thyroid-related eye disease (TED) has been thoroughly
 57 investigated. It has been suggested that the fibroblasts, rather than the myocytes, are the main
 58 targets of the auto-immune attack. Focal infiltrations of lymphocytes in the orbit are similar in
 59 structure to those found in the thyroid gland of patients with auto-immune thyroiditis, the so-
 60 called thyroid associated lymphoid tissue (TALT) phenotype (1). TALT is thought to be
 61 involved in a local production of thyroid auto-antibodies and generation of auto reactive T-
 62 cells, which starts in the draining lymph nodes. Because the eye muscles share their draining
 63 lymph nodes with the thyroid (2), this notion of redistribution of lymphocytes expressing
 64 thyroid specific homing receptors (addressins) from lymph nodes to thyroid (3) may similarly
 65 involve the eye muscles. The pathophysiology of ophthalmopathy could thus be explained by
 66 recognition of a shared auto-antigen such as TSH-receptor peptides, heat shock proteins or the
 67 “kDa protein” by a specific homing pattern of the thyroid-reactive lymphocytes to the eye
 68 muscles (shared addressins/adhesion molecules) (1).

69 With progression of the eye disease and over production of ground substance and
 70 collagen in the endomysium and perimysium, the muscle fibres become progressively
 71 compressed and finally atrophie (1). This muscle fibre destruction is thought to be followed
 72 by the production of eye muscle autoantibodies as occurs following cardiac infarction which
 73 leads to the production of autoantibodies to heart muscle cells (1). The main eye muscle
 74 antigen appears to be calsequestrin although the corresponding serum antibodies are detected
 75 in only a third of patients with active ophthalmopathy in one study, but more often in others
 76 (4). The notion that ophthalmopathy is triggered is by eye muscle autoantibodies appears
 77 unlikely and the ophthalmopathy seems more closely related to the thyroiditis rather than
 78 being an independent entity (5). Thus, ophthalmopathy should logically be associated with

79 both Graves' disease and Hashimoto's thyroiditis. Indeed, there is good evidence that
80 orbitopathy does occur in patients with Hashimoto's thyroiditis (1, 6), although less often.

81 Age, gender and smoking have been clearly associated with the presence of
82 ophthalmopathy in patients with Graves' disease, but these associations were not investigated
83 in patients with Hashimoto's thyroiditis (7). Age and gender were found to influence the
84 severity of ophthalmopathy in a cohort of 101 patients attending a thyroid-eye clinic, which
85 included 9 patients with Hashimoto's thyroiditis (8). Graves' disease was shown to be milder
86 and Hashimoto's thyroiditis less prevalent in aged patients, probably because of a reduced
87 responsiveness of the aged thyrocyte to autoantibodies (9). Several other studies confirmed
88 that male patients with Graves' disease are more at risk for a severe ophthalmopathy (10).

89 The effect of smoking in patients with Hashimoto's thyroiditis is controversial, mainly
90 because only a few studies are available (11-13). More recent studies suggest that smokers
91 with either Graves' disease or Hashimoto's thyroiditis are more at risk for ophthalmopathy (6,
92 14). Some studies have been reported to imply an association between smoking and negative
93 TPO antibodies in patients with Hashimoto's thyroiditis (11) while negative TPO antibodies
94 in patients with Graves' thyroiditis was found to be a risk factor for ophthalmopathy (15). The
95 severity of Graves' disease in male and young patients has been suggested by their low rate of
96 remission, requiring a more aggressive management (16). Moreover, higher titres of
97 circulating thyroid autoantibodies were found in elderly patients with Graves' disease
98 compared to young patients (17). This study is the first to investigate all the various known
99 and possible risk factors for ophthalmopathy in a cohort of 105 patients with Hashimoto's
100 thyroiditis.

101

102 CLINICAL SUBJECTS AND METHODS

103

104 Clinical subjects

105 The study was a retrospective analysis of data from medical records of patients who
106 attended the endocrinology clinic at Nepean Hospital (Kingswood, NSW, Australia) between
107 2007 and 2012. Only patients with a confirmed and recent (not yet treated, or treated for less
108 than 3 months) diagnosis of Hashimoto's thyroiditis were selected. Only data from the first
109 visit were used and only complete data sets for the analysis were included. This was a
110 retrospective audit which was approved by the Nepean Hospital Human Ethics committee.
111 Informed written consent was not required and there were no consent forms.

112 Serum vitamin D, Tg antibodies and TPO antibodies

113 All laboratory results were generated by the same institution namely, Barratt and
114 Smith Pathology Sydney, using commercial kits. A patient with a serum level of vitamin D
115 below 51nmol/l was considered as having a vitamin D deficiency (lab normal range: 51-
116 140nmol/l). Patients who received vitamin D supplementation before their first visit were
117 excluded from the study. The laboratory cut off titres for thyroid peroxidase (TPO) antibody
118 was < 10 IU/ml (range 0-120) and for thyroglobulin (Tg) antibody < 20 IU/ml (range 0-90).

119 Statistical analysis

120 For continuous variables, means and standard deviations or medians and interquartile
121 ranges were computed. Normality was tested using the Kolmogorov Smirnov and the Shapiro
122 Wilks tests. Binary logistic regression analysis was performed to identify the predicting
123 factors. Four separate logistic regression calculations were performed to identify risk factors
124 for 1) ophthalmopathy 2) upper eyelid retraction (UER) 3) Nunery score (18) and 4) Clinical
125 Activity Score (CAS)(19). A p value less than 0.05 was considered statistically significant.
126 The statistical software IBM SPSS Statistics, version 19, was used for all analyses.

127

128 RESULTS

129 Demographics of the study population

130 The number of patients included in this study is 105, comprising 97 women and 8
131 men. The lowest age of the studied cohort was 14 years and the highest was 82. The median
132 age was 48. The patients were then categorised as “old” or “young” when their age was above
133 or equal/below 50. Among the selected patients, 25% were smokers, 89.2% were positive for
134 TPO antibodies, 45.7% were positive for Tg antibodies and 46% were vitamin D deficient.
135 The calculated median value of Tg and TPO serum levels was approximately 400 IU/ml in
136 our cohort. This value was then chosen as a threshold to categorise patients as having low or
137 high TPO or Tg serum levels. Those details are summarised in Tables 1 and 2.

138 Risk factors for ophthalmopathy in patients with Hashimoto’s thyroiditis

139 After exclusion of patients with incomplete data set, 86 patients were included in the
140 binary logistic regression analysis. Ageing has a statistically significant protective impact
141 against the development of ophthalmopathy in patients with Hashimoto’s thyroiditis. Each
142 additional year of age decreases the risk for ophthalmopathy by 5.4%. On the other hand,
143 smoking has a statistically significant negative effect, exposing patients with Hashimoto’s
144 thyroiditis to a 5.5 times higher risk for ophthalmopathy. Neither gender, nor vitamin D
145 deficit, nor serum levels of TPO or Tg antibodies were predictive for ophthalmopathy
146 development in patients with Hashimoto thyroiditis (Table 3).

147 Risk factors for upper eyelid retraction in patients with Hashimoto thyroiditis

148 After exclusion of patients with incomplete data set, 73 patients were included in the
149 binary logistic regression analysis (table 4). Age, gender, smoking and vitamin D deficit do

not appear to contribute to the development of UER in patients with Hashimoto's thyroiditis. The risk for UER is significantly decreased when patients have high serum levels of TPO antibodies. A similar trend was observed with high serum levels of Tg antibodies, but this was not statistically significant.

Risk factors for severe ophthalmopathy in patients with Hashimoto's thyroiditis

After exclusion of patients with incomplete data set, 33 patients were included in the binary logistic regression analysis. The type 2 of Nunery classification was not observed in any of our patients. Combined Nunery type 1+2 ophthalmopathy, i.e. congestive ophthalmopathy and ocular myopathy together, as a more severe ophthalmopathy, was then compared to type 1. None of the investigated risk factors were found to influence the development of type 1+2 vs. type 1 ophthalmopathy (Table 5).

Risk factors for more active ophthalmopathy in patients with Hashimoto thyroiditis

After exclusion of patients with incomplete data set, 69 patients were included in the binary logistic regression analysis (Table 6). Among the investigated risk factors, only gender showed a statistically significant influence on the activity of ophthalmopathy in patients with Hashimoto thyroiditis. Male patients are indeed 18 times more at risk for a highly active ophthalmopathy. However, it has to be noted that men are relatively underrepresented in our cohort, although it is well known that autoimmune thyroiditis is more prevalent in women than in men with a ratio up to 10:1(20).

DISCUSSION

Ophthalmopathy in patients with Hashimoto thyroiditis has the same demographics, clinical characteristics and severity and activity scores as in patients with Graves' disease

(21). It is therefore not surprising that in our cohort, the observed effect of age, gender, smoking and TPO antibodies on ophthalmopathy in patients with Hashimoto thyroiditis is similar to the previously described effect of these factors on ophthalmopathy in patients with Graves' disease (7, 10, 16, 17).

Many publications have shown differences in the immune system of young and old subjects, but the results of these studies were overall discordant (22). However, there is a general agreement on the existence of so-called immunosenescence involving mainly the T cells (22-25). For example, some authors have shown that elderly individuals are more susceptible to rheumatoid arthritis (25) although others (23) suggest that this might not apply to other autoimmune diseases. Interestingly, it has been demonstrated experimentally that the efficiency of self-tolerance is reduced in young individuals, who are therefore more susceptible to the development of multiple sclerosis after exposure to an environmental trigger (26). The direct mechanisms of self-tolerance are clonal anergy and apoptotic elimination of self-specific T cells (25). Prevention and reversal of autoimmune diabetes have been experimentally achieved through the elimination of self-reactive T cells (27). It has also been demonstrated that the proportion of regulatory T cells, which maintain peripheral tolerance by suppressing auto-reactive T cells (25), increases with age (28). It would be interesting to compare the proportion of regulatory T cells in patients with ophthalmopathy vs. those without ophthalmopathy in patients with Graves' disease and Hashimoto's thyroiditis. These regulatory T cells were shown to originate from the thymus, which plays a crucial role in the prevention of autoimmune thyroiditis. Steroid hormones regulating thymic function are also thought to influence the development of autoimmune thyroiditis which peaks for different post-menopausal women populations and declines in later years for both Hashimoto's thyroiditis and Graves' disease (29).

Autoimmune thyroiditis is less prevalent in men but once they have it, they are more at risk to develop more severe ophthalmopathy than women (8,10,30). This greater risk for severe autoimmunity in men was explained, in the case of systemic lupus erythematosus, by a higher cumulative genetic load required for men to develop the disease. An amino acid change in the HLA-DR β 1 chain, from glutamine into arginine at position 74, was shown to confer a strong risk for both Graves' disease and Hashimoto's thyroiditis (31). It was demonstrated that this amino acid change results in a unique structure of the HLA-DR pocket which becomes able to bind auto-antigenic peptides and present them efficiently to T cells. A similar mechanism was found to play a major role in the genetic susceptibility to type 1 diabetes (HLA-DQ β -Asp57). Several other susceptibility genes for autoimmune thyroiditis have been identified, such as cytotoxic T lymphocyte-associated protein 4 (CTLA-4) gene of which normal function is to suppress T cell activation, and the CD40 gene which has a key role in B cell activation and antibody secretion. Besides these immune regulatory genes, there are thyroid-specific genes such as the thyroglobulin (Tg) gene, a variant of which (W1999R) was found to have a strong genetic interaction with HLA-DR β -Arg74, which increases the susceptibility to Graves' disease. Most of these susceptibility genes were shown to have individually a weak effect and it has been estimated that more than 20 different variants are required to develop autoimmune thyroiditis (32). It would be interesting to compare the genetic load required for men to develop autoimmune thyroiditis and ophthalmopathy Vs. women.

TPO and Tg antibodies are markers for Hashimoto thyroiditis and may play a pathogenetic role. TPO antibodies are detected in more than 90% of the patients with Hashimoto thyroiditis, while Tg antibodies are detected in a lower proportion. TPO antibodies production was shown to be genetically determined (33). We hypothesise that the observed association between high levels of circulating TPO and the absence of UER is more

likely to be due to shared genetic determinants which are still to be identified and would regulate both features in an opposite manner.

Vitamin D deficiency was shown to have a role in the development of autoimmune diseases (type1 diabetes and Addison's disease) whereas the stimulation of vitamin D receptor (VDR), which is expressed by tolerogenic dendritic cells, promotes self-tolerance. An association between VDR gene polymorphisms and Hashimoto thyroiditis has been identified in different populations (33). In our cohort, vitamin D deficiency does not influence ophthalmopathy.

The detrimental effect of smoking on ophthalmopathy is well established for Graves ophthalmopathy (GO). It increases by 4 times the risk for developing or worsening GO. We observed a similar effect of smoking on the development of ophthalmopathy in patients with Hashimoto's thyroiditis. Several mechanisms have been suggested as an explanation for the adverse effect of cigarette smoking on GO. One of these mechanisms is tissue hypoxia which induces the secretion of large amounts of glycosaminoglycans (GAGS) by cultured orbital fibroblasts. The accumulation of these GAGS in orbital tissues contributes to the swelling of extraocular muscles and orbital fat. More directly, exposure to tobacco products has been shown to upregulate the expression of HLA-DR in orbital fibroblasts and exposure to cigarette smoke increases the secretion of pro-inflammatory cytokines by these cells, which then differentiate *in vitro* into adipocytes. *In vivo*, current smokers have a larger extraocular muscles volume but not more orbital fat when compared to non-smokers (34). We think that these mechanisms could also apply for Hashimoto's associated eye disease.

Another risk factor for ophthalmopathy is the newly identified r3832814 polymorphism in the CASQ1 gene whose absence is associated with an increase risk of ophthalmopathy in patients with Graves' disease (Lahooti *et al* submitted for publication).

In conclusion, similarly to Graves' disease, ophthalmopathy in patients with Hashimoto's thyroiditis is less frequent with aging, more active in men and more prevalent in smokers. For the first time, we found an association between high levels of TPO antibodies and the absence of UER which is often the main eye symptom in these patients. This finding may lead to new research avenues to investigate the genetic determinants and the pathophysiology of UER.

Disclosure Statement

The authors declare that no conflict of interest exists.

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362 Table 1. Contingency tables for the investigation of risk factors for ophthalmopathy and upper
363 eyelid retraction in patients with Hashimoto's thyroiditis

	¹ TED	No TED	Total	² UER	No UER	Total
Age>50years	9	38	47	13	22	35
Age=<50years	20	38	58	25	25	50
Men	3	5	8	5	3	8

Women	26	71	97	33	44	77
Smoker	9	12	21	8	8	16
Non smoker	20	64	84	30	39	69
Vitamin D deficiency	10	31	41	12	18	30
No vitamin D deficiency	15	33	48	23	23	46
High ³ TPO>400	15	43	58	15	31	46
Low TPO<400	11	22	33	16	11	27
Negative TPO	3	8	11	6	3	9
High ⁴ Tg>400	6	15	21	3	11	14
Low Tg<400	9	18	27	10	12	22
Negative Tg	14	43	57	25	24	49

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365 ¹ TED= thyroid related eye disease

366 ² UER=upper eyelid retraction

367 ³ TPO = thyroid peroxidase

368 ⁴ Tg = thyroglobulin

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370 Table 2. Contingency tables for the investigation of risk factors for ophthalmopathy subtypes

371 and severity in patients with Hashimoto's thyroiditis

	¹ Nunery	Nunery	Total	² CAS	CAS 3,4,5	Total
	1	1&2		0,1,2		
Age>50years	10	0	10	28	4	32

Age= $\leq$ 50years	25	3	28	38	10	48
Men	5	1	6	3	4	7
Women	30	2	32	63	10	73
Smoker	7	1	8	12	4	16
Non smoker	28	2	30	54	10	64
Vitamin D deficiency	13	0	13	20	7	27
No vitamin D deficiency	18	2	20	38	6	44
High ^{3} TPO >400	19	1	20	38	6	44
Low TPO <400	13	1	14	20	5	25
Negative TPO	3	1	4	6	3	9
High ^{4} Tg >400	7	0	7	13	1	14
Low Tg <400	12	0	12	18	2	20
Negative Tg	16	3	19	35	11	46

372 ¹ Nunery (18) 1(congestive ophthalmopathy), 2(ocular myopathy)

373 ² CAS = Clinical Activity Score (19)

374 ³ ^{3} TPO = thyroid peroxidase

375 ⁴ Tg = thyroglobulin

376 Table 3. Identification of predicting variables for ophthalmopathy in patients with

377 Hashimoto's thyroiditis

Variables	<i>p</i> Value	Exp(B)	95% C.I.for EXP(B)	
			Lower	Upper
Age	.014	.946	.904	.989

Smoking	.010	5.515	1.500	20.269
Gender	.217	3.028	.521	17.579
Vitamin D	.635	1.005	.984	1.027
¹ TPO antibodies	.569	1.000	1.000	1.000
² Tg antibodies	.616	1.000	1.000	1.000

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379 ¹ TPO = thyroid peroxidase

380 ² Tg = thyroglobulin

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390 Table 4. Identification of predicting variables for ophthalmopathy in patients with

391 Hashimoto's thyroiditis

Variables	<i>p</i> Value	Exp(B)	95% C.I. for EXP(B)	
			Lower	Upper

Age	.118	.965	.923	1.009
Smoking	.167	2.683	.662	10.866
Gender	.396	2.189	.359	13.357
Vitamin D	.836	1.002	.982	1.022
¹ TPO antibodies	.010	.258	.091	.726
² Tg antibodies	.130	.290	.059	1.440

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393 ¹ TPO = thyroid peroxidase

394 ² Tg = thyroglobulin

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404 Table 5. Identification of predicting variables for severe ophthalmopathy in patients with

405 Hashimoto's thyroiditis

Variables	<i>p</i> Value	Exp(B)	95% C.I.for EXP(B)	
			Lower	Upper

Age	.642	.968	.843	1.111
Smoking	.332	8.272	.116	589.361
Gender	.999	**	**	**
Vitamin D	.299	1.072	.940	1.223
1TPO antibodies	.987	.973	.038	24.718
2Tg antibodies	.999	**	**	**

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407 **Value could not be calculated

408 ¹ TPO = thyroid peroxidase

409 ² Tg = thyroglobulin

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420 Table 6. Identification of predicting variables for highly active ophthalmopathy in patients

421 with Hashimoto's thyroiditis

Variables	<i>p</i> Value	Exp(B)	95% C.I. for EXP(B)	
			Lower	Upper
Age	.314	.969	.912	1.030
Smoking	.082	4.194	.836	21.043
Gender	.013	18.173	1.852	178.289
Vitamin D	.229	.981	.951	1.012
TPO antibodies	.376	.543	.140	2.101
Tg antibodies	.208	.182	.013	2.572

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