

Research paper

Vitamin D deficiency may be a risk factor for ophthalmopathy in patients with Graves' hyperthyroidism but not Hashimoto's thyroiditis

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ABSTRACT

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43 **Background:** Apart from its key role in bone metabolism vitamin D plays a role in
44 immune function, cancer prevention and autoimmunity. Vitamin D deficiency (serum
45 level < 25 nmol/L) and insufficiency (25-50 nmol/L) are very common in the West
46 Sydney area of Australia. Vitamin D deficiency may also play a role in the development
47 of ophthalmopathy in patients with thyroid autoimmunity

48 **Methods:** We have studied a possible relationship between serum vitamin D level and
49 ophthalmopathy in patients with Graves' disease and Hashimoto's thyroiditis. We studied
50 37 patients with Graves' disease and 69 with Hashimoto's thyroiditis before any vitamin
51 D replacement.

52 **Results:** Overall, 70% of patients with Graves' disease and 86% of those with
53 Hashimoto's thyroiditis were vitamin D deficient or insufficient. Sixty-one % of Graves'
54 patients who were vitamin D deficient and 75% of those who were vitamin D insufficient
55 had ophthalmopathy, defined as a NOSPECS class of two or more, compared to only 27%
56 of patients who were vitamin D replete, the difference being significant. Eleven % of
57 patients with Hashimoto's thyroiditis who were vitamin D deficient and 22% of those who
58 were vitamin D insufficient had ophthalmopathy, which was generally mild and often
59 manifest as isolated upper eyelid retraction, compared to 20% of patients who were
60 vitamin D replete, which was not significant. There was no close correlation between
61 vitamin D status and either the activity or severity of the ophthalmopathy, for either
62 Graves' disease or Hashimoto's thyroiditis.

63 **Conclusions:** Vitamin deficiency is significantly associated with ophthalmopathy in
64 patients with Graves' hyperthyroidism but not Hashimoto's thyroiditis. The finding that a

normal serum vitamin D level decreased the risk of developing ophthalmopathy in patients with Graves' hyperthyroidism, but not Hashimoto's thyroiditis, suggests that the pathogenesis of the eye changes in the two disorders may be different. While the significance of the findings needs to be addressed in a prospective study it seems that vitamin D deficiency is another risk factor for ophthalmopathy in patients with Graves' hyperthyroidism.

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72 INTRODUCTION

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Although best known for its role in calcium absorption and bone metabolism, there is increasing evidence that vitamin D plays a major role in immune function, cancer prevention and autoimmunity (1). In particular, vitamin D deficiency (serum level (< 25 nmol/L) and insufficiency (25-50 nmol/L) are linked to several autoimmune disorders including; rheumatoid arthritis (2, 3), multiple sclerosis (4), type 1 diabetes (5), lupus and Graves' disease (6 - 8). Vitamin D deficiency is common in high latitude countries because of lack of sunlight, especially during the long winters, with correspondingly increased prevalences of autoimmune disorders compared to climates that are more temperate (9, 10). It is estimated that 80% of people living in the West Sydney area are also vitamin D insufficient or deficient, mainly as a result of an over-effective skin cancer prevention programme, lack of sunlight sun exposure and life style issues and because the food is not enriched with vitamin D in Australia. In the present study we have addressed a possible relationship between serum vitamin D status and ophthalmopathy in patients with Graves' disease and Hashimoto's thyroiditis. We showed a significant correlation between vitamin D deficiency or insufficiency and ophthalmopathy in patients with Graves' hyperthyroidism but not Hashimoto's thyroiditis.

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CLINICAL SUBJECTS AND METHODS

Clinical subjects

The patients attended the thyroid clinics at Nepean Hospital, Australia. Local Ethics Committee approval was obtained for this retrospective study and consent forms were not required. The study concerned;

- i) 37 patients, 3 males and 34 females aged 21 - 81 (mean age 47 yr) with Graves' hyperthyroidism
- ii) 69 patients, 5 males and 64 females aged 14 - 76 (mean age 52 yr), with Hashimoto's thyroiditis and,
- iii) 82 patients, 10 males and 72 females aged 24 – 82 (mean age 60 yr), with multi nodular goitre (MNG), as non autoimmune controls

The diagnoses of the various thyroid disorders were determined on the basis of standard clinical criteria and confirmed by thyroid function testing, immunological tests and real-time thyroid ultrasonography. Any associated ophthalmopathy was classified in respect to; Werner NOSPECS classes (11), Nunery types I (without restrictive myopathy) or II (with restrictive myopathy) (12) and as a modified clinical activity score (CAS) (0-12), based on that described by Mourits *et al.* (13) which takes into account signs and symptoms of upper eyelid disease that is often the main features of the eye changes of Hashimoto's thyroiditis. For the purpose of the study ophthalmopathy was taken as a NOSPECS class of two or more, regardless of clinical activity score, in the case of the eye changes of Hashimoto's thyroiditis.

METHODS

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116 **Vitamin D measurement:**

117 Serum vitamin D, as 25-OH Vitamin D (vitamin D2) was measured by Barratt &
 118 Smith Pathology using the DiaSorin CLIA Method 2, with a normal range of 50-145
 119 nmol/L. We defined vitamin D status according to recommendations outlined in the
 120 position statement “Vitamin D and adult bone health in Australia and New Zealand” (14)
 121 where mild deficiency (here “insufficiency”) is defined as a serum level of 25-OH
 122 Vitamin D (DiaSorin CLIA Method 2) (“vitamin D”) of 25-50 nmol/L, moderate
 123 deficiency as a level of 12.5 – 25 nmol/L, severe deficiency as a serum level < 12.5
 124 nmol/L and “replete” as a level > 50 nmol/L.

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126 **Statistical analyses**

127 Serum vitamin D levels in patients with Graves’ disease and Hashimoto’s
 128 thyroiditis, with and without ophthalmopathy, and control subjects with multi nodular
 129 goitre, were compared using X^2 and odds ratio using statistical package Graph Pad
 130 version 3. A two tailed p value of < 0.05 was considered significant

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132 **RESULTS**

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134 The relationship between vitamin D status, thyroid diagnosis and any
 135 associated ophthalmopathy was assessed at the first visit, before any vitamin D
 136 replacement, in patients with Graves’ disease, Hashimoto’s thyroiditis and, as non
 137 autoimmune control, MNG. We addressed the possibility that vitamin D deficiency was
 138 more common in patients with Graves’ ophthalmopathy than in those with no eye signs
 139 (Graves’ hyperthyroidism) and whether a similar association was found in patients with

Hashimoto's thyroiditis. Overall, 48% of patients with Graves' disease were vitamin D deficient, 22% were insufficient and 30% were replete, compared to 39%, 47% and 14%, respectively, of patients with Hashimoto's thyroiditis and 51%, 37% and 12% respectively, of patients with MNG. When compared to control patients with MNG, the prevalences of normal serum levels of vitamin D was significantly increased in patients with Graves' disease [χ^2 test, $p = 0.048$, odds ratio $p =$] but not Hashimoto's thyroiditis (χ^2 test, $p = 0.258$, odds ratio 0.819 (confidence interval 0.319 – 2.12) $p = 0.679$ (table 1). Sixty-one % of Graves' patients who were vitamin D deficient and 75% of those who were vitamin D insufficient had ophthalmopathy (defined as NOSPECS class of 2 or more), compared to only 27% of patients who were vitamin D replete (Table 2). This difference was significant by odds ratio [5.03 (confidence interval 1.06 – 23.8) $p=0.033$] and showed a trend to significance by χ^2 test ($P = 0.084$). Eleven % of patients with Hashimoto's thyroiditis who were vitamin D deficient and 22% of those who were vitamin D insufficient had ophthalmopathy, which was generally mild and often manifest as isolated upper eyelid retraction (UER), compared to 20% of patients who were vitamin D replete. This difference was not significant [χ^2 test $p = 0.539$, odds ratio 0.816 (confidence interval 0.150 – 4.43) $p = 0.813$] (table 3). There was no close correlation between vitamin D status and either the activity or severity of the ophthalmopathy, for either Graves' disease or Hashimoto's thyroiditis (results not shown).

DISCUSSION

The role of vitamin D in cancer, immune function and autoimmunity is of great current interest. It is now recognised that low serum levels of vitamin D3 is a risk factor

for abnormal immune function including the development of autoimmune disease and worse cancer prognosis. Vitamin D is also important in the context of the development of osteoporosis, a major health issue in older female populations. To summarise the main findings of the study; we have confirmed the very high prevalences of vitamin D deficiency and insufficiency in the West Sydney area which includes the Blue Mountains area, where iodine deficiency is also a recognised problem (15). We did not study normal subjects, but the age and sex ranges of patients with Graves' disease, Hashimoto's thyroiditis and MNG can be taken as representative of the population in this area. The overall prevalence of vitamin D deficiency or insufficiency was approx. 80% in the study population. The higher prevalence of normal vitamin D levels in patients with Graves' disease than those with Hashimoto's thyroiditis and MNG may reflect the slightly younger population of patients who develop the former disorder. The main aim of the study was to address a possible relationship between vitamin D deficiency and ophthalmopathy in patients with Graves' hyperthyroidism. We showed that a high prevalence of vitamin D deficiency or insufficiency in patients with Graves' ophthalmopathy compared to those without eye signs, but not in patients with Hashimoto's thyroiditis who have mainly mild ophthalmopathy manifest as UER and other eyelid signs.

Overt ophthalmopathy is common in patients with Graves' disease while UER with or without generally mild ophthalmopathy are found in about 25% of patients with Hashimoto's thyroiditis (16). TSH-receptor antibodies have been implicated in the development of ophthalmopathy, particularly congestive subtype (17, 18). However, there is also a strong correlation between antibodies against the skeletal muscle protein, calsequestrin and both extraocular muscle and upper eyelid muscle in patients with Graves' disease and Hashimoto's thyroiditis (19-22). The observed relationship between vitamin D deficiency/insufficiency and ophthalmopathy suggests that the pathogenesis of

the two disorders is different. Patients studied here were seen at the first visit; since deficient and sufficient patients were treated with vitamin D we were not able to obtain follow-up data in respect to any possible change in the ophthalmopathy. However, we postulate that vitamin D repletion may play a role in the improvement in eye signs. In the future we would measure serum calsequestrin and collagen XIII antibodies in larger numbers of patients, correlating levels with initial vitamin D status and with supplementation. In conclusion; while the significance of the findings needs to be addressed in prospective studies before and after vitamin D replacement, it seems possible that vitamin D deficiency is, along with smoking and stress, another risk factor for ophthalmopathy in patients with Graves' hyperthyroidism.

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Table 1 Relationship between serum Vitamin D level and thyroid diagnosis in patients with autoimmune and non-autoimmune thyroid disorders in the West Sydney area of Australia.

Vitamin D status at first visit	Thyroid diagnosis		
	Graves' disease` (n=37)	Hashimoto's thyroiditis (n=69)	Multinodular goitre (n=82)
Deficient (serum vitamin D2 < 25 nmol/L)	18 (48%)	27 (39%)	43 (51%)
Insufficient (serum vitamin D2 25-50 nmol/L)	8 (22%)	32 (47%)	29 (37%)
Normal (serum vitamin D2 > 50 nmol/L)	11 (30%)	10 (14%)	10 (12%)
¹ X ²	6.06	2.70	
P-Value	0.048	0.258 NS	
¹ Odds Ratio	0.328 (confidence interval 0.125 – 0.863)	0.819 (confidence interval 0.319 – 2.12)	
P-Value	0.020	0.678 NS	

¹ Statistical analyses refer to comparison of prevalences of normal vitamin D levels in patients with autoimmune thyroid disorders (Graves' disease, Hashimoto's thyroiditis) Vs control patients with multi nodular goitre. NS = not significant

Table 2 Relationship between serum vitamin D level and ophthalmopathy in patients with Graves' hyperthyroidism in the West Sydney area of Australia

Vitamin D status at first visit	¹ Ophthal	No Ophthal
Deficient (serum vitamin D2 < 25 nmol/L) (n=18)	11 (61%)	7 (39%)
Insufficient (serum vitamin D2 25-50 nmol/L) (n= 8)	6 (75%)	2 (25%)
Normal (serum vitamin D2 > 50 nmol/L) (n= 11)	3 (27%)	8 (73%)
² X ²	4.951	
P-Value	0.084 NS	
Odds ratio	5.03 (confidence interval 1.06 – 23.8)	
P-Value	0.033	

¹ Ophthal = ophthalmopathy

² Statistical analyses refer to differences between ophthal vs. no ophthal determined using X² tests and from odds ratios. NS=not significant.

Table 3 Relationship between serum vitamin D level and ophthalmopathy in patients with Hashimoto's thyroiditis in the West Sydney area of Australia

Vitamin D status at first visit	Ophth.	No Ophth.
Deficient (serum vitamin D2 < 25 nmol/L) (n=27)	3 (11%)	24 (89%)
Insufficient (serum vitamin D2 25-50 nmol/L) (n=32)	7 (22%)	25 (78%)
Replete (serum vitamin D2 > 50 nmol/L)	2 (20%)	8 (80%)
χ^2	1.236	
P-Value	0.539 NS	
Odds ratio	0.816 (confidence interval 0.150 – 4.43)	
P-Value	0.813 NS	

¹ Ophthal = ophthalmopathy

² Statistical analyses refer to differences between ophthal vs. no ophthal determined using χ^2 tests and from odds ratios. NS=not significant.