

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

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48 **ABSTRACT**

49 **Aims:** Apart from its key role in bone metabolism vitamin D plays a role in immune
50 function, cancer prevention and autoimmunity induction. Vitamin D insufficiency (25-50
51 nmol/L) and deficiency (serum level < 25 nmol/L) are very common in the West Sydney
52 and Blue Mountains areas of Australia. Vitamin D deficiency may also play a role in the
53 development of ophthalmopathy in patients with thyroid autoimmunity

54 **Methods:** We have studied a possible relationship between serum vitamin D level and
55 ophthalmopathy in patients with Graves' disease and Hashimoto's thyroiditis. We studied
56 37 patients with Graves' disease and 69 with Hashimoto's thyroiditis at their first clinical
57 visit, before any vitamin D replacement.

58 **Results:** Overall, 70% of patients with Graves' disease, 86% of those with Hashimoto's
59 thyroiditis and 88% of patients with Multinodular goitre control group were vitamin D
60 deficient or insufficient. Sixty-one % of Graves' patients who were vitamin D deficient and
61 75% of those who were vitamin D insufficient had ophthalmopathy, defined as a
62 NOSPECS class of two or more, compared to only 27% of patients who were vitamin D
63 replete, the difference being significant. Thirty nine % of Graves' patients who were
64 vitamin D deficient and 25% of those who were vitamin D insufficient had no
65 ophthalmopathy, compared to 73% of patients who were vitamin D replete. Eleven % of
66 patients with Hashimoto's thyroiditis who were vitamin D deficient and 22% of those who
67 were vitamin D insufficient had ophthalmopathy, which was generally mild and often
68 manifest as isolated upper eyelid retraction, compared to 20% of patients who were
69 vitamin D replete, which was not significant. There was no close correlation between
70 vitamin D status and either the activity or severity of the ophthalmopathy, for either
71 Graves' disease or Hashimoto's thyroiditis.

72 **Conclusions:** Vitamin deficiency is significantly associated with ophthalmopathy in
73 patients with Graves' hyperthyroidism but not Hashimoto's thyroiditis. The finding that a
74 normal serum vitamin D level was associated with a decreased prevalence of

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. It appears that Graves' patients with ophthalmopathy have a greater propensity to vitamin D deficiency as compared to Graves' patients without ophthalmopathy. 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.

Keywords; vitamin D, Graves' disease, Hashimoto's thyroiditis, ophthalmopathy, upper eyelid retraction

1. INTRODUCTION

Although best known for its role in calcium absorption and bone metabolism, there is increasing evidence that vitamin D plays a role in immune function, cancer prevention and autoimmunity development (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 (6) and Graves' disease (7, 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, such as rheumatoid arthritis, compared to climates that are more temperate (9, 10). It is estimated that 80% of people living in the West Sydney and Blue Mountains areas 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.

2. 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. We have not used the VISA system in this study as this is a very complicated protocol for the characterisation of the eye and orbital changes. 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.

VITAMIN D MEASUREMENT:

Serum vitamin D, as 25-OH Vitamin D (vitamin D2) was measured by Barratt & Smith Pathology using the DiaSorin CLIA Method 2, with a normal range of 50-145 nmol/L. We defined vitamin D status according to recommendations outlined in the position statement "Vitamin D and adult bone

health in Australia and New Zealand" (14) where mild deficiency (here "insufficiency") is defined as a serum level of 25-OH Vitamin D (DiaSorin CLIA Method 2) ("vitamin D") of 25-50 nmol/L, moderate deficiency as a level of 12.5 – 25 nmol/L, severe deficiency as a serum level < 12.5 nmol/L and "replete" as a level > 50 nmol/L.

STATISTICAL ANALYSES

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

3. RESULTS AND DISCUSSION

The relationship between vitamin D status, thyroid diagnosis and any associated ophthalmopathy was assessed at the first visit, before any vitamin D replacement, in patients with Graves' disease, Hashimoto's thyroiditis and, as non autoimmune control, MNG. We addressed the possibility that vitamin D deficiency was more common in patients with Graves' ophthalmopathy than in those with no eye signs (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 prevalence of normal serum levels of vitamin D was significantly increased in patients with Graves' disease [χ^2 test, *P* = 0.04, odds ratio 0.328 (confidence interval 0.125-0.863 *P* = 0.02] but not Hashimoto's thyroiditis (χ^2 test, *P* = 0.25, odds ratio 0.819 (confidence interval 0.319 – 2.12) *P* = 0.67 (table 1).

Sixty-one % of patients with Graves' disease 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*=0.03] and showed a trend to

significance by X^2 test ($P = 0.08$). Thirty nine % of Graves' patients who were vitamin D deficient and 25% of those who were vitamin D insufficient had no ophthalmopathy, compared to only 73% of patients who were vitamin D replete. It appears that Graves' patients with ophthalmopathy have a greater propensity to vitamin D deficiency as compared to Graves' patients without ophthalmopathy. 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 [X^2 test $P = 0.53$, odds ratio 0.816 (confidence interval 0.150 – 4.43) $P = 0.81$] (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).

Vitamin D deficiency is a major risk factor for the development of osteomalacia and osteoporosis. However, vitamin D also plays an important role in normal immune function, cancer prevention and autoimmunity and it is now recognised that low serum levels of vitamin D3 are associated with abnormal immune function including the development of autoimmune disease such as Graves' disease (22), multiple sclerosis and type 1 diabetes, and worse cancer prognosis. Here we have addressed a possible relationship between vitamin D deficiency and ophthalmopathy in patients with Graves' disease and Hashimoto's thyroiditis. 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 and patients with MNG do not have thyroid autoimmunity. The overall prevalence of vitamin D deficiency or insufficiency was approx. 80% in the study population. The increased prevalence of normal vitamin D levels in patients with Graves' disease compared to those with Hashimoto's thyroiditis and MNG may reflect the slightly younger population of patients who develop the former disorder. Other studies have shown that Graves' disease is associated with vitamin D deficiency (7, 8) which may have been masked in the present study by the overall high prevalence of low levels in the great majority of subjects living in this area.

192 The main aim of the study was to address a possible relationship between vitamin D deficiency
193 and ophthalmopathy in patients with Graves' hyperthyroidism. We showed that a high prevalence
194 of vitamin D deficiency or insufficiency in patients with Graves' ophthalmopathy compared to
195 those without eye signs, but not in patients with Hashimoto's thyroiditis who have mainly mild
196 ophthalmopathy manifest as UER and other eyelid signs. The mechanism for the putative effect of
197 vitamin D deficiency on the development of ophthalmopathy is unknown but likely to reflect the
198 complex interrelationship between this and other risk factors for the eye disorder such as smoking
199 status, genetics, stress and other environmental factors that could be studied using an animal
200 model for Graves' disease. Many immune cells, including T lymphocytes and dendritic cells,
201 express the Vitamin D receptor (VDR), stimulation of which has been shown to enhance
202 tolerogenicity. One possibility is one can postulate that modulation of the VDR, which would be
203 dependent on existing levels of Vitamin D in the environment, alters the function of the T
204 regulatory cells and thus promotes the development of autoimmune disorders including
205 ophthalmopathy.

206 Overt ophthalmopathy is common in patients with Graves' disease while UER with or without
207 generally mild ophthalmopathy are found in about 25% of patients with Hashimoto's thyroiditis
208 (16). TSH-receptor antibodies have been implicated in the development of ophthalmopathy,
209 particularly the congestive subtype (17, 18). However, there is also a strong correlation between
210 antibodies against the skeletal muscle protein calsequestrin and both extraocular muscle and
211 upper eyelid muscle in patients with Graves' disease and Hashimoto's thyroiditis, respectively (19-
212 22). The observed relationship between vitamin D deficiency/insufficiency and ophthalmopathy
213 suggests that the pathogenesis of the two disorders may be different. We have not studied our
214 patients following vitamin D supplementation so we are unable to document any improvement in
215 the ophthalmopathy with treatment.

216 The relationship between vitamin D availability and ophthalmopathy could be determined from the
217 study of different population groups. We are unaware of any studies addressing the prevalence
218 and severity of ophthalmopathy in patients with Graves' disease in high latitude countries
219 compared to those in equatorial regions of the world in relation to availability of vitamin D from the
220 sunlight and food. While it is likely that vitamin D deficiency does play a role in the pathogenesis
221 of the eye disorder, and that repletion would improve the thyroid eye signs, this needs to be

addressed in a large multi centre study of patients with Graves' disease and Hashimoto's thyroiditis, correlating eye changes with serum calsequestrin, collagen XIII, TSHR antibody and vitamin D levels. We would also address the roles of other risk factors such as smoking, stress, severity of the thyroid eye disease in patients with Graves' ophthalmopathy.

4. CONCLUSION,

While the significance of the findings needs to be addressed in prospective studies before and after vitamin D replacement, vitamin D deficiency appears to be, along with smoking , 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 ¹ (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	0.04	0.258 NS	
¹ Odds Ratio	0.328 (confidence interval 0.125 – 0.863)	0.819 (confidence interval 0.319 – 2.12)	
P-Value	0.02	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	0.08 NS	
Odds ratio	5.03 (confidence interval 1.06 – 23.8)	
P-Value	0.03	

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	Ophthal.	No Ophthal.
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%)
² X 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 X² tests and from odds ratios. NS=not significant.