Research Article

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Biomarkers in Medicine

Vitamin D levels are associated with epistaxis severity and bleeding duration in hereditary hemorrhagic telangiectasia

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Aim: To explore the association between vitamin D levels and mild versus severe epistaxis, as well as the overall epistaxis severity score (ESS) in patients with hereditary hemorrhagic telangiectasia. **Patients & methods:** A retrospective chart review of 198 patients was performed to explore the relationship between vitamin D levels and the ESS. Vitamin D levels were also compared with those with mild epistaxis to those with severe epistaxis. **Results:** A significant difference was found between patient's vitamin D levels and their associated ESS and duration of epistaxis. Patients with mild epistaxis had higher levels of vitamin D than patients with severe epistaxis. **Conclusion:** Vitamin D is associated with features of hereditary hemorrhagic telangiectasia including ESS, bleeding time and epistaxis severity.

First draft submitted: 18 July 2017; Accepted for publication: 19 December 2017; Published online: 14 March 2018

Keywords: biomarker • epistaxis • hereditary hemorrhagic telangiectasia • Osler–Weber–Rendu • vitamin D

Heredity hemorrhagic telangiectasia (HHT) is an autosomal dominant disorder characterized by epistaxis, solid organ arteriovenous malformations and a unique pattern of oral/dermal telangiectases [1]. These malformations are characterized by direct arteriovenous connections [2] and can affect multiple organs including skin, lungs, brain, gastrointestinal, nasal mucosa and liver [3]. In certain tissues/organs, nasal, GI mucosa and brain in particular, these malformations are prone to bleed spontaneously or with minimal trauma. In other locations, lungs and liver in particular, complications typically result not from bleeding but by shunting of blood *per se* through these high flow lesions [1]. Severity of epistaxis, the most common symptom of HHT, is currently best measured using a validated tool, the epistaxis severity score (ESS) [4].

These vascular malformations are most often due to mutations in two genes (*ENG;* HHT1) and (*ACVRL1*; HHT2) that are involved in the TGF-β and BMP9 signaling pathway [5]. Mutations in this pathway ultimately leads to malformed blood vessels and subsequent features of HHT [6]. Some patients who are clinically diagnosed with HHT do not have a mutation in one of the known HHT genes. Although phenotypic differences have been reported between genetic subtypes of HHT, significant 'intra-familial variation is reported, both in terms of the site and number of telangiectases or arteriovenous malformations, and severity of related symptoms' [1]. Based on this observation that the primary disease-causing mutation in an HHT gene does not fully explain the spectrum of phenotypic expression seen within families, other modifiers of the HHT phenotype are predicted. Potential biomarkers of disease expression and severity are thus being explored.

Vitamin D (cholecalciferol) has shown to be protective in cardiovascular disorders. In a recent study by Gibson *et al.* the authors sought to discover drugs that can be repurposed to treat cerebral cavernous malformation (CCM) [7]. CCM has some similarity to HHT in that they are both hereditary diseases where malformed endothelial cells lead to poorly functioning blood vessels and excessive bleeding. In this study, loss-of-function of *CCM2*, one of the underlying genetic causes, was used in a mouse-model to exhibit the lesions of CCM. Two biomarkers were

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identified, vitamin D (cholecalciferol) and tempol (a scavenger superoxide) for further exploration. Both vitamin D_3 and tempol reduced CCM lesions in a mouse model of human CCM disease [7]. A second study regarding vitamin D and CCM found that patients with chronically aggressive disease had significantly lower 25- (OH) vitamin D than those who did not have features of chronically aggressive disease [8].

Research into the connection between vitamin D and endothelial cell function has been reviewed extensively by Dalan *et al.*, where they showed that vitamin D deficiency is found to be associated with endothelial cell dysfunction and cardiovascular diseases [9]. Notably, results that are similar to the aforementioned CCM studies, an earlier study by Zhong *et al.*, also showed the protective effects of vitamin D_3 and superoxides on endothelial cells [10]. Specifically, they found that vitamin D induces a dose and time-dependent increase in the vitamin D receptor expression. It also induces upregulation of VEGF and increased expression of CuZn superoxide dismutase in endothelial cells [10]. These are associated in the endothelial repair pathway. It has also been shown that vitamin D_3 has modulating effects on the TGF-β pathway – the pathway associated with mutated genes in both CCM and HHT [7,11].

Due to past research exposing the protective nature of vitamin D in mouse-models and as a marker of disease severity in diseases that are similar to HHT, its role providing protective effects on endothelial cells and its function in the TGF-β pathway, a retrospective chart review study was performed to see if there are links between vitamin D levels and the phenotypic, clinical expression of HHT.

Patients & methods

This is a retrospective chart review study on patients with HHT seen at the University of Utah HHT Center for Excellence, which sees approximately 125 patients with this rare disorder per year. The ESS has been routinely recorded since 2010. ESS was first developed by Dr Hoag in 2010, as a standardized method to measure epistaxis severity [12]. ESS is an increasing scale from 0–10 based on the answers to six questions involving: frequency, duration, intensity (typically gushing vs not gushing), whether they have sought medical attention for their epistaxis, if they are anemic due to their epistaxis and if they have received a red blood cell transfusion for epistaxis. Levels of 25-(OH) vitamin D have been recorded for several years. Values below 30 ng/ml are considered as deficient in vitamin D according to University of Utah's ARUP lab standards. An IRB approved protocol (00039582) allows for retrospective chart review studies of patients seen at the University of Utah HHT Center.

A total of 198 adult patients (18+), seen at the HHT Center in the last three years were analyzed. Patients were included in analysis if they had ESS and vitamin D level recorded. Fifty-six percent of the patients were female and 44% were male. Ages ranged from 18 to 86, with an average and median age of 51. Two separate analysis were performed.

For the first analysis, patients were divided into four groups based on their 25 (OH)-vitamin D levels: vitamin D $\leq$10 ng/ml (n = 7), vitamin D 11–20 ng/ml (n = 18), vitamin D_3 21–30 ng/ml (n = 43) and vitamin D_3 $\geq$31 ng/ml (n = 42). These groups were chosen based on prior research establishing various clinical manifestations that occur at these levels of deficiency. The number of participants in each group are the participants that had all qualifying data (e.g., vitamin D level, ESS) required for analysis. These four groups were then compared via a one-way analysis of variance to their ESS as well as the aforementioned components that make up the ESS. Patients were excluded if they have had Young's nasal closure surgery performed, nasal laser/cautery or septal dermoplasty within the past 2 years, due to the potential to skew ESSs.

The second analysis divided patients into three groups: mild, moderate and severe epistaxis. Mild epistaxis is characterized by an ESS <3 and no interventions such as laser or cautery (n = 26). Severe epistaxis is characterized by an ESS >7, Young's procedure or previous septal dermoplasty (n = 37). Moderate epistaxis is characterized by anything that falls between mild and severe epistaxis (n = 135). An unpaired (two sample) t-test was performed between mild and severe epistaxis, and compared based on their 25-(OH) vitamin D levels.

These data were extracted and compiled into a unified Excel spreadsheet. Microsoft Excel version 15.13.4 and MetaboAnalyst version 3.2.2 from McGill University were used to perform the statistical analyses [13]. Statistical tests were considered significant if $p < 0.05$.

Results

Vitamin D levels & total ESS

A one-way analysis of variance was calculated based on patients' vitamin D levels and their associated ESS. Vitamin D_3 levels were divided into four groups: $\leq$10, 11–20, 21–30 and $\geq$31 ng/ml. Values >30 ng/ml were considered

Table 1. Average epistaxis severity score for each vitamin D level.

Vitamin D level ng/ml	Epistaxis severity score	SD	95% CI
<10	6.19	1.86	1.95 (4.24–8.14)
11–20	3.68	2.15	1.14 (2.54–4.82)
21–30	4.68	2.16	0.63 (4.05–5.31)
>31	3.39	2.09	0.68 (2.71–4.07)

SD: Standard deviation.

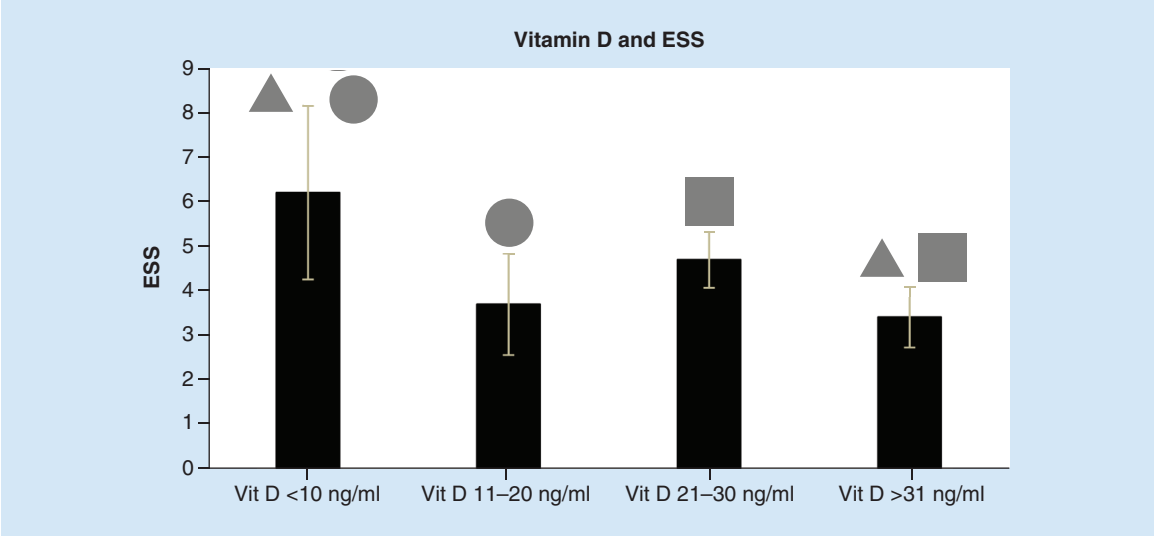

Figure 1. Vitamin D levels and their associated average epistaxis severity score, with error bars indicating the 95% CI.
Matching shapes represent groups that have statistically significant epistaxis severity scores between Vit D groups ($p < 0.05$).
ESS: Epistaxis severity score; Vit D: Vitamin D.

to be 'normal' and therefore was considered the maximum group of vitamin D_3 for the calculations. A significant difference between the four levels of vitamin D and their ESS was found, (F [3,105] = 3.67, p = 0.015). *Post-hoc* analysis using Fisher's least significant difference method showed results were significant between the following groups: vitamin D ≤10 ng/ml & vitamin D 11–20 ng/ml, vitamin D 21–30 ng/ml & vitamin D ≥31 ng/ml, vitamin D ≤10 ng/ml & vitamin D ≥31 ng/ml. See Table 1 & Figure 1 for ESS averages in each group and their associated SDs and 95% CIs. Shapes in Figure 1 denote the groups for which *post-hoc* analysis showed statistical significance.

Vitamin D levels & epistaxis duration

Epistaxis duration, one component of the ESS, ranges from an average of less than 1 min to greater than 30 min. Averages for the range of minutes in each category were used to allow for a continuous variable. There was a significant difference of the epistaxis duration based on the levels of vitamin D (F [3,106] = 3.62, p = 0.016]). *Post-hoc* analysis using Fisher's least significant difference method showed results were significant between the following groups: vitamin D ≤10 ng/ml & vitamin D 11–20 ng/ml, vitamin D ≤10 ng/ml and vitamin D ≥31 ng/ml. See Table 2 & Figure 2 for epistaxis duration averages in each group and their associated SDs and 95% CIs. We did not see a statistically significant difference on epistaxis frequency, intensity or whether or not they sought medical attention in regards to vitamin D levels.

Mild versus severe epistaxis & vitamin D

The aforementioned results exhibited a trend where very low levels of vitamin D (≤10 ng/ml) were often statistically significant when compared with healthy levels of vitamin D (≥30 ng/ml). Due to this significance we divided patients into mild or severe epistaxis (requirements for group designation were mentioned in the Patients &

Table 2. Average epistaxis duration in minutes for each vitamin D level.			
Vitamin D level ng/ml	**Epistaxis duration (minutes)**	**SD**	**95% CI**
<10	13.93	5.79	4.84 (9.09–18.88)
11–20	6.56	6.21	3.58 (3.01–10.14)
21–30	9.51	7.47	2.57 (6.94–12.08)
>31	5.46	3.87	1.35 (4.11–6.81)
SD: Standard deviation.			

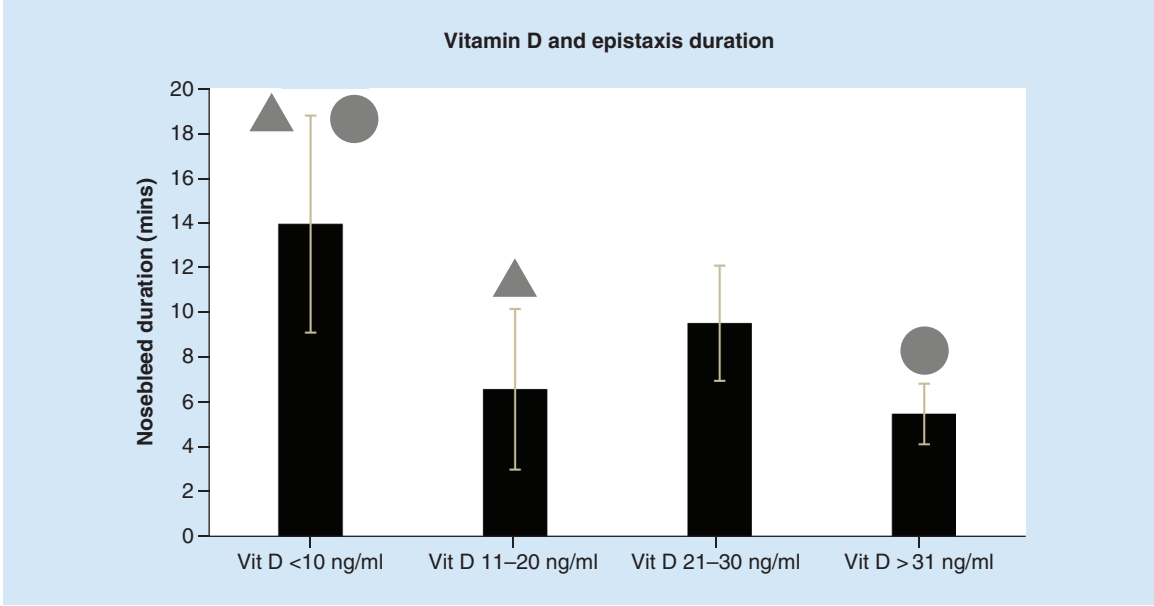

Figure 2. Vitamin D levels and their associated average epistaxis duration in minutes, with error bars indicating the 95% CI.
Matching shapes represent groups that have statistically significant epistaxis severity scores between vitamin D groups ($p < 0.05$).
Vit D: Vitamin D.

Table 3. Average vitamin D level for mild versus severe epistaxis.			
Epistaxis severity	**Vitamin D ng/ml**	**SD**	**95% CI**
Mild epistaxis	34.6	9.72	3.24 (31.6–37.84)
Severe epistaxis	17.8	6.42	2.59 (15.21–20.39)
SD: Standard deviation.			

methods section) and compared them based on their vitamin D levels. The results showed that those with milder epistaxis had statistically higher levels of vitamin D (M = 34.6, SD = 9.72) than those who had severe epistaxis (M = 17.8 ng/ml; SD = 6.42 t[61] = -8.23, $p < 0.001$). See Table 3 & Figure 3 for epistaxis duration averages in each group and their associated SDs and 95% CIs. Those who had mild epistaxis had vitamin D levels above the current recommended amount, whereas those who had severe epistaxis had vitamin D levels below.

Discussion

Patients with HHT often endure numerous clinical manifestations of their disease that create significant distress for them. Epistaxis is a common burden that many patients have to deal with on a daily basis. Therefore, exploration into factors that are associated with epistaxis should help advance patient care and management. This retrospective chart review explores the link between vitamin D and one significant clinical manifestation of HHT: epistaxis. This assessment was undertaken due to recent research linking vitamin D to vascular diseases (e.g., CCM), endothelial cell function and the TGF-β pathway. We found a statistically significant difference among the varying levels of

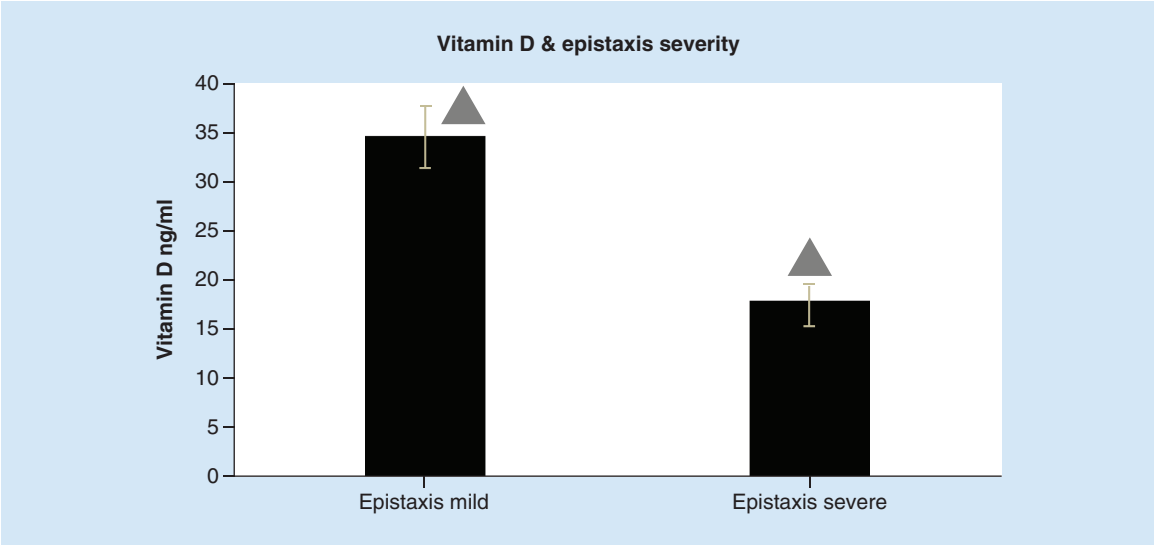

Figure 3. Mild versus severe epistaxis and their associated vitamin D levels, with error bars indicating the 95% CI. Shape represents statistical significance between the two groups ($p < 0.001$).

vitamin D and their associated ESS. *Post-hoc* analyses revealed statistically significant results between patients with very low levels of vitamin D (≤10 ng/ml) levels compared with those with healthy levels of vitamin D (≥30 ng/ml).

Among the six components of the ESS, the epistaxis duration showed a statistically significant difference ($p = 0.016$) among the varying levels of vitamin D as well. *Post-hoc* analyses revealed that having very low levels of vitamin D_3 (≤10 ng/ml) result in epistaxis duration that are statistically different from vitamin levels that are higher (11–20 and ≥31 ng/ml). This is particularly interesting as vitamin D has been shown to stabilize endothelial cells of blood vessels, indicating a potential link between vitamin D and decreased bleeding time in patients who are predisposed to already weak blood vessels. Vitamin D levels between the extremes of very low levels of vitamin D (≤10 ng/ml) and healthy levels of vitamin D (≥31 ng/ml) do not follow the anticipated trend (see Figures 1 & 2) – this may be due to the middle groups being too similar in nature. In both of the analysis, neither middle group is statistically significant from the other, potentially indicating their similarity. Interestingly, when the two middle groups (11–20 and 21–30 ng/ml) are averaged together, they follow the expected trend.

There was no direct Pearson's r correlation between the total ESS and vitamin D levels when they were treated as continuous variables rather than in groups. This may be due to the fact that the ESS is a significantly smaller scale than serum vitamin D, and are thus not comparable. In addition, many people's ESS and vitamin D levels vary among different times or exposures in their lives – variables that may alter the ESS and vitamin D may be geographical location, sun exposure and time of year. In addition, patients with anemia due to blood loss may predispose them to feel fatigued and subsequently have less sun exposure. Supplementation and diet should not, hypothetically affect any of the results, as the proposed hypothesis uses total serum 25-(OH) vitamin D in the analyses. Two main variables that we thought would pose the biggest threat are gender and age; however, when analyses were performed to see if there was a significant difference between these groups (e.g., those who had mild epistaxis compared with those who had severe epistaxis) there was no significant difference of age or gender between the two groups. Despite our best efforts to exclude individuals that may have altered ESS because of treatment, there are many aforementioned factors as well as other potentially undiscovered biomarker modifiers that may allow for significant phenotypic variability. Nonetheless, these results indicate that there is difference in epistaxis among patients with varying vitamin D levels, which warrants further exploration.

Conclusion & future perspective

Sixty-two percent of the total patients with recorded vitamin D levels were deficient, indicating a need for further research on the clinical implication of low vitamin D levels in patients with HHT. The results are important due to the large number of patients who are deficient and the potential effects vitamin D may have on overall ESS, duration of epistaxis and severity of epistaxis. Regardless of the unknown precise biological role of vitamin D in

patients who have HHT genetic mutations, we find the results to underscore the need for a continued search for biomarkers to reduce the severity of the disease and improve quality of life in these patients.

Past research has deemed vitamin D to be an important regulator in cardiovascular diseases, endothelial cell dysfunction and the TGF-β pathway. These findings in conjunction with our chart review indicates a future need to explore the role of vitamin D among patients with HHT in more detail. A study of vitamin D treatment of mouse models of HHT may be an interesting scientific endeavor, as vitamin D has been shown to be protective in mouse models of CCM. Currently in HHT, invasive and expensive treatments such as VEGF-inhibitors, laser, cautery and nasal closures are used to inhibit excessive nasal bleeding. It would be interesting to see if future studies can hone in on the protective role of vitamin D on epithelial cells to see if increasing vascular integrity of current blood vessels, rather than eliminating malformed vessels can be a potential treatment.

As has been previously established in other cardiovascular disorders, maintenance of proper vitamin D levels in patients with HHT should help overall patient health, regardless of our findings. We find this research helps bring awareness to the fact that there may be undiscovered biomarkers that are modulating clinical manifestations of HHT. Further research is needed to establish causality between vitamin D and epistaxis; however, we hope our findings will help researchers ask more questions in this realm.

Summary points

Background

- Vitamin D has been shown to be protective in mouse models and as a marker of disease severity in diseases that are similar to heredity hemorrhagic telangiectasia (HHT), to provide protective effects on endothelial cells and to modulate the TGF-β pathway.

Patients & methods

- A retrospective chart review of 198 patients with HHT was analyzed for their epistaxis severity score (ESS) and vitamin D levels.

Results

- Patients with mild epistaxis had significantly higher vitamin D levels than those with severe epistaxis. Patients with different vitamin D levels had different ESSs and differences among bleeding duration.

Conclusion

- This retrospective chart review highlights differences among patients with HHT with regard to their ESS and epistaxis duration. This article attempts to promote questioning of vitamin D levels in patients with HHT; however, further research is needed to establish causality.

Financial and competing interests' disclosure

The authors have no relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript. This includes employment, consultancies, honoraria, stock ownership or options, expert testimony, grants or patents received or pending, or royalties.

No writing assistance was utilized in the production of this manuscript.

Ethical conduct of research

There was appropriate institutional review board approval (00039582) and maintenance of protected patient information.

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