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Original article

Serum vitamin D levels correlate with the presence and histological grading of colorectal adenomas in peri and postmenopausal women

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SUMMARY

Background & aims: Vitamin D is known to modulate immune function and proliferation. Higher vitamin D [25(OH)D_3] serum levels have been reported to have protective effects on adenoma detection and colorectal cancer (CRC) development and survival.

Methods: This retrospective cohort study included 315 peri and post-menopausal women submitted to opportunistic colorectal and osteoporosis screening at the gynaecology outpatient clinic of a tertiary medical centre between 2004 and 2015. Colonoscopy findings were correlated with 25(OH)D_3 and PTH serum levels, and subsequently adjusted in a multivariate logistic regression model. Confounding factors included demographic and colorectal risk factors, pharmacological therapies and bone densitometry metrics.

Results: A total of 77 lesions were identified in 66 patients. Vitamin D insufficiency (<30 ng/mL) and deficiency (<20 ng/mL) were identified in 79.4% and 35.2% of patients, respectively. In univariate analysis, lower levels of 25(OH)D_3 were associated with polyp, adenoma and advanced adenoma detection. After adjusting for confounders, an association with polyps could not be observed, but a trend towards a negative correlation with adenoma detection was found (adjusted OR: 0.96; 95% CI 0.92–1.00; $p = 0.083$). Regarding advanced adenoma detection, 25(OH)D_3 (adjusted OR: 0.86; 95% CI 0.77–0.97; $p = 0.013$) proved to be an independent predictive factor. No association was found between 25(OH)D_3 levels and lesion detection site.

Conclusion: The association of 25(OH)D_3 serum levels with colorectal lesions seems to be restricted to adenomatous lesions and is influenced by histological grading. Vitamin D may be a valuable biomarker for optimization of risk stratification in group-specific CRC screening protocols.

1. Introduction

Approximately 1.3 million colorectal cancers (CRC) are diagnosed every year, mostly in developed countries. CRC is now the third most frequent cancer in men, and the second in women [1]. Surveillance programs have shown to significantly reduce incidence and mortality by as much as 30%, and colonoscopy remains the gold standard by reducing, not only mortality, but also disease incidence through detection and excision of pre-cancerous lesions [2–4]. Surveillance protocols assess patient's risk and establish follow-up intervals using criteria based on individual (e.g. age, family history of CRC) and endoscopic (e.g. lesion number, size and histology) features. However, the persistence of a high rate of missing lesions and interval cancers suggests a window for risk assessment improvement.

Vitamin D is a pro-hormone thought to play a part in processes that extend beyond its classical calcium homeostasis role. Vitamin

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D receptor (VDR) is known to modulate immune function and proliferation at the mucosal level, and is expressed throughout the colonic mucosal [5].

Recently, vitamin D [25(OH)D_3] has been put forward as a potential biomarker for colorectal adenomas and cancer. In the past decade, studies consistently reported an inverse association between vitamin D serum levels and risk of colorectal adenoma. An approximately 7–12% risk reduction was estimated for every 10 ng/mL increase of 25(OH)D_3 [6–10]. However, this association was not observed for hyperplastic polyps, probably reflecting the lower malignant potential of these lesions [7]. Additionally, in most studies the protective effect of a higher 25(OH)D_3 was stronger – and sometimes even exclusive – in female patients [8].

The risk of CRC is also inversely associated with vitamin D serum levels [11–16]. A large meta-analysis including approximately 1 million patients corroborated this finding [17]. But vitamin D serum levels also correlate with survival, as has been demonstrated in studies where pre-diagnostic 25(OH)D levels were shown to be independent positive predictors of overall survival in colorectal cancer [18–21].

The pathophysiology mechanisms underlying these effects have also been studied in both animal models and humans. Murine models demonstrate an anti-neoplastic effect of 1,25-(OH)D_3, achieved through proliferation inhibition, differentiation induction, and apoptosis [22–25]. In human colonic mucosa, supplemental calcium and vitamin D3 intake produce an increase in calcium and vitamin D receptors, APC/β-catenin ratio, E-cadherin expression and apoptosis [26–29], a decrease in inflammatory cytokines (TNFα, IL-1β; IL-6) [30], and modulation of DNA oxidative damage and growth factor expression (TGFβ1 and TGFα) [31,32]. Moreover, in patients with CRC, 25(OH)D_3 serum levels correlate with higher intratumoral immune cell reaction, and hence greater host immunological surveillance, which has been proposed to contribute to the improved survival observed in these patients [33].

Therefore, evidence supports vitamin D as a strong biomarker for colorectal adenomas and cancer. This study aimed to explore the association between serum levels of 25(OH)D_3 and parathyroid hormone (PTH) with colorectal polyps and their histological grading. The analysis was confined to peri and post-menopausal women, as they seem to be the subset of patients where the strongest protective effect is observed [34].

2. Materials & methods

2.1. Study design and patients

This was a single centre retrospective study of a cohort of 532 peri or post-menopausal women enrolled in an opportunistic CRC screening program at the Gynaecology and Reproduction Medicine outpatient clinic of Hospital Santa Maria, which is a tertiary academic referral centre. From 2004 to 2015 three hundred and fifteen women (n = 315) were simultaneously screened for CRC and osteoporosis. Demographics and colorectal-specific risk factors were collected from medical records.

2.2. CRC screening

Based on age and family history of CRC, patients were submitted to opportunistic CRC screening by videocolonoscopy. Patients with an unacceptable bowel preparation (Boston bowel preparation score ≤ 1 in any segment) or incomplete left colon screening were excluded from the analysis. Data on number, size and histology were retrospectively collected. Lesions were graded in four categories: 1) no lesions; 2) hyperplastic polyps; 3) non-advanced adenoma (low grade dysplasia or <10 mm); and 4) advanced lesion (high grade dysplasia, villous morphology or >1 cm lesion) or adenocarcinoma. If more than one lesion was identified in the same patient, the most advanced lesion was considered. Lesion location was classified according to distal (rectum, sigmoid or descending colon) and proximal (transverse or ascending colon) segments.

2.3. Osteoporosis screening

Osteoporosis screening was performed in women aged 65 or older and in any younger women with equivalent or higher fracture risk. Serum levels of 25(OH)D_3, parathyroid hormone (PTH) and calcium were collected, as well as data from dual-energy X-ray absorptiometry (DEXA) scans (including lumbar and femur T-scores, and Z-scores). When a patient had two or more measurements, the mean value was considered. The Institute of Medicine estimates for vitamin D minimum and the Endocrine Society reference range for clinical practice were used to define insufficiency (<30 ng/mL) and deficiency (<20 ng/mL) [35,36].

2.4. Demographic data and risk factors

Data on demographic (age, ethnicity), gynaecologic (age of menarche, menopause, parity) and colorectal risk factors (body mass Index [BMI] [37], family history of colorectal cancer [FHCRC], smoking status) were retrieved from patients' medical records. Data on confounding pharmacological therapies was also collected, including calcium/vitamin D supplementation, use of oestrogen modulators and bisphosphonates therapy.

2.5. Statistical analysis

The study was initially powered to detect a 3 ng/mL difference in mean 25(OH)D_3 serum levels for the primary endpoint of adenoma detection, assuming a 30% adenoma detection rate, 80% power and 95% confidence interval, in a predicted sample size of 318 patients. Sample size was however restricted by the retrospective and opportunistic nature of the study.

Chi-square test or Fischer Exact test were used to analyse categorical variables. Quantitative variables were compared using T-test for independent samples or Mann–Whitney non-parametric test when appropriate. A binary logistic regression model with entry method was used to compute the multivariate analysis. The following confounding factors were adjusted: age; Body Mass Index (BMI); weight; smoking status; first degree relatives with CRC (FHCRC); age of menarche; 25(OH)D_3 and PTH serum levels; month of blood collection year, and calcium/vitamin D supplementation. Statistical analysis was performed using IBM SPSS Statistics v24®. All statistical tests were two-sided, and P values of less than 0.05 were considered statistically significant.

3. Results

3.1. Population characteristics

Characteristics of this study cohort are depicted in Table 1. The 315 women included had a mean age of 63 ± 7 years, and the vast majority (95.2%) were Caucasian. Most patients were post-menopausal (94.9%). Sixteen patients (5.1%) were peri Mean BMI was 29.0 ± 4.8 kg/m^2, and most women were overweight (40.1%) or obese (38.8%). Active smoking was reported in 33 (10.3%) patients, and a positive first-degree family history of CRC, in 26 (8.3%) patients.

Table 1
Baseline demographic and clinical characteristics of included patients. Continuous variables are described by means ± SD. Categorical variables are described by number (%).

Patient characteristics	
Patients	N = 315
Age (yr.)	62.98 ± 7.15
Ethnicity, n (%)	
White	300 (95.2)
African	14 (4.4)
Asiatic/Indian	1 (0.3)
Body mass index (kg/m^2)	29.01 ± 4.75
Weight (kg)	69.87 ± 12.05
Smoking	
Yes	33 (10.5)
No	282 (89.5)
FHCRC	
Yes	26 (8.3)
No	289 (91.7)
Vitamin D/calcium supplementation[a]	
Yes	191 (62.8)
No	113 (37.2)
Oestrogen therapy[a]	
Yes	176 (57.9)
No	128 (42.1)
Bisphosphonate therapy[a]	
Yes	53 (17.4)
No	251 (82.6)
Menopausal status	
Postmenopausal	299 (94.9)
Perimenopausal	16 (5.1)
Age of menopause (yr.)	50.19 ± 4.73
Age of menarche (yr.)	13.25 ± 1.65
Parity	
≥1	297 (94.3)
0	18 (5.7)
25(OH)D_3 (ng/mL)	25.53 ± 9.78
PTH (pg/mL)[b]	50.10 ± 22.25
Calcium (mg/dL)	9.64 ± 0.43
Lumbar spine T-score	−1.16 ± 1.52
Femur T-score	−0.45 ± 3.46
Colonoscopy findings, n (%)	
Grading:	
No polyps	249 (79)
Hyperplastic polyps	20 (6.3)
Adenomas (LGD, <1 cm)	36 (11.4)
Advanced Lesion (HGD, villous, >1 cm or adenocarcinoma)	10 (3.1)
Location:	
Proximal	48 (72.7)
Distal	13 (19.7)
Missing data	3 (4.5)

[a] Missing data on 11 patients (n = 304).
[b] Missing data on 9 patients (n = 306).

3.2. CRC screening

Screening was performed by videocolonoscopy, which identified a total of 77 lesions/polyps in 66 patients, corresponding to a 20.9% polyp detection rate. Distribution by histological grading was as follows: hyperplastic polyp, n = 20 (6.3%); LGD adenoma, n = 36 (11.4%); advanced lesion, n = 8 (2.5%); and invasive adenocarcinoma, n = 2 (0.6%). Adenoma detection rate was 14.5%. Considering location, the highest-grade lesion was detected in the distal colon for 48 (72.7%) patients, and in the proximal colon for 13 (19.7%) patients. Videocolonoscopy findings are shown in Table 1.

3.3. Vitamin D, PTH and osteoporosis screening

As foreseen, a relevant number of women exhibited a DEXA t-score compatible with osteopenia (42.3%) or osteoporosis (19.6%). More than half of patients reported taking calcium/vitamin D supplements (62.8%) or oestrogen replacement therapy (57.9%), and 17.4% of patients were on bisphosphonate therapy.

Mean 25(OH)D_3 serum level was 24.0 ± 7.6 ng/mL. Seventy eight percent of patients had some degree of vitamin D insufficiency. Vitamin D deficiency (<20 ng/mL) was present in 107 (34%) patients, whereas vitamin D insufficiency (20–30 ng/mL) was present in 140 (44%) patients. Mean PTH serum level was 48.4 ± 18.6 ng/mL. Both 25(OH)D_3 ($p = 0.016$) and PTH ($p = 0.029$) serum levels displayed a significant seasonal variation, with higher values reported in late spring and summer months (Fig. 1). Serum 25(OH)D_3 showed a nonlinear association with PTH levels (Fig. 2).

3.4. Hyperplasic polyp, adenoma and advanced lesion detection

Detection of any type of lesion was associated with a positive family history of CRC (16.7% vs 6.0% – unadjusted OR: 2.23; $p = 0.005$) as well as with active smoking status (16.7% vs 8.8%, unadjusted OR: 1.71; $p = 0.073$). There was also a trend towards higher weight values (adjusted OR: 1.05 95% CI 0.99–1.11; $p = 0.066$) and lower menarcheal age (adjusted OR: 0.82 95% CI 0.67–1.01; $p = 0.065$). After adjusting for confounding factors, however, only a positive FHCRC (OR: 3.90 95% CI 1.48–10.31; $p = 0.006$) and smoking (OR: 2.49, 95% CI 1.05–8.39; $p = 0.038$) were significantly associated with overall lesion detection (Table 2).

Regarding specifically the detection of any type of adenomatous lesions, vitamin D was the only variable for which an association was observed. A tendency towards lower levels of serum 25(OH)D_3 was observed in affected patients. However, although this association was statistically significant at univariate analysis (22.9 ± 9.6 vs 25.6 ± 9.0; $p = 0.045$), significance was lost after adjusting for confounding factors (OR: 0.96, 95% IC 0.92–1.00; $p = 0.083$).

Restricting the analysis to the detection of only advanced lesions exposed a clear association with lower 25(OH)D_3 serum levels (18.3 ± 7.4 vs 25.8 ± 9.8; $p = 0.020$) and with higher PTH serum levels (65.9 ± 26.6 vs 49.6 ± 21.9; $p = 0.044$). After adjusting with a binary logistic regression model, association was maintained only for vitamin D (OR: 0.86, 95% CI 0.77–0.96; $p = 0.009$). In the same model, FHCRC (OR: 10.26, 95% CI 1.19–88.19; $p = 0.034$) and calcium/vitamin D supplementation (OR: 9.06, 95% CI 1.23–64.24; $p = 0.027$) were also correlated with detection of advanced lesions.

As seen in Fig. 3 a negative logarithmic correlation was observed between 25(OH)D_3 serum levels and the histology grading of each lesion detected. A plateau was observed among patients without lesions and among those with only hyperplastic polyps. Low grade adenomas and advanced lesions displayed an increasing tendency towards lower 25(OH)D_3 serum levels.

Lesion location (distal vs proximal colon) was not associated with any of the variables studied (Table 3). Even considering higher grade polyps, no association with 25(OH)D_3 or PTH serum levels was observed.

4. Discussion

We aimed to explore the association between vitamin D and PTH with colorectal adenoma's risk assessment in peri and postmenopausal women. The main finding of our study was the consistent negative association between Vitamin D levels and colorectal adenomatous lesions. Furthermore, this association was influenced by lesion severity, with higher histological grades exhibiting a stronger negative association with vitamin D serum levels.

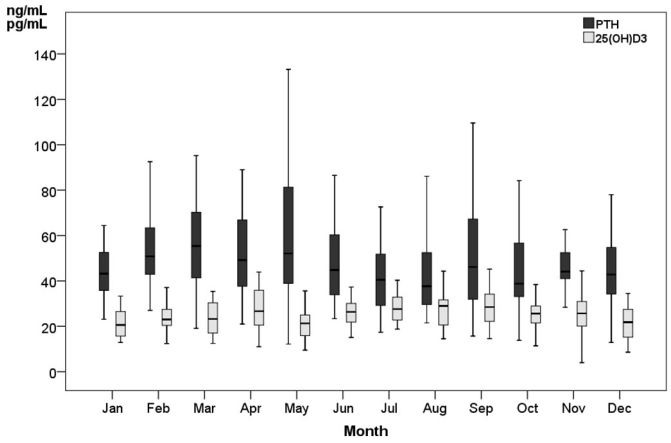

Fig. 1. Mean serum levels of vitamin D and PTH throughout months of the year. A significant seasonal variation was observed (ANOVA, Vitamin D p = 0.016; PTH p = 0.029).

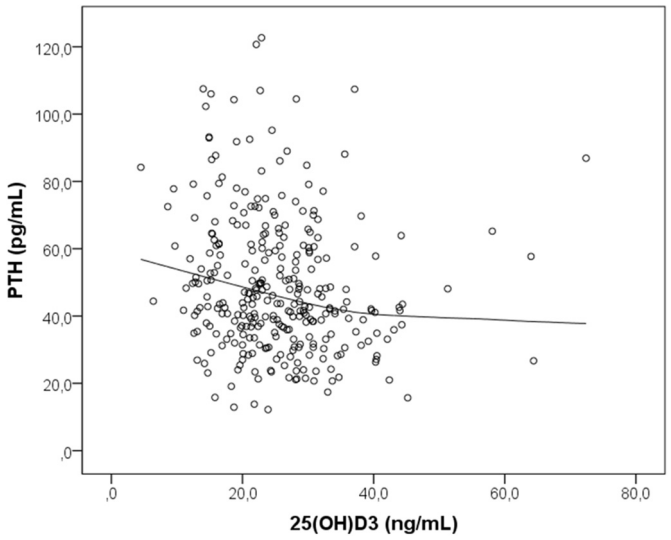

Fig. 2. Distribution of serum 25(OH)D_3 and PTH levels. LOES adjustment line (95% CI).

The selection of the specific group of peri and post-menopausal women relied on previously reported data suggesting these patients have the strongest protective effect of higher vitamin D levels concerning colorectal neoplastic lesions [34]. Then again, we recognize that this is a particularly vitamin D deficiency-prone subset of patients. In our cohort, three quarters of women were vitamin D insufficient (<30 ng/mL), and one third was vitamin D deficient (<20 ng/mL). These values closely resemble the ones previously published regarding other populations [38,39] but they may not reflect a true state of deficiency or inadequate intake. Indeed some authors argue that daily requirements corresponding to a 25(OH)D_3 serum level of 20 ng/mL may suffice for the majority of the population [36,40]. Still, this cut-off relates primarily to bone health and may not apply to extra-skeletal effects of vitamin D where higher serum vitamin D levels may contribute or at least serve as a biomarker for general wellbeing and lower risk of disease [41–44].

Aside from the debate surrounding the concept of deficiency, the low 25(OH)D_3 serum levels observed in our patients certainly have multiple contributing factors: increased age [45], higher BMI [46], higher incidence of osteoporosis [45], and decreased oestrogen levels as a result of post-menopausal status [47]. Additionally, these women are also more prone to be receiving specific therapies, as calcium/vitamin D, oestrogens or bisphosphonates, which impact as well on vitamin D and PTH serum levels. Although such confounding factors were addressed and explored in this study, it was not possible to account for some other potentially important variables, such as decreased physical activity and sun exposure.

Colorectal cancer screening detected a total of 77 colonic lesions in 66 patients. This corresponds only to a 20.9% polyp detection and to a 14.5% adenoma detection rate (ADR). This ADR is considerably below the 20% cut-off for female patients, which is currently the standard of quality in colonoscopy [48–50]. Furthermore it is unexpectedly low if we consider this to be a high-risk population due to the with high prevalence of vitamin D insufficiency. Two possible explanations may be a high variability among endoscopists performing the procedures, and difficulty in achieving high quality bowel preparations in these advanced age, overweight patients. As this seems to be a significant limitation of this study, we may also speculate that, since three quarters of these patients were vitamin D insufficient, a higher ADR would most probably support and strengthen our findings. On the other hand, one should note that ADR does not reflect a true adenoma burden since it does not translate a quantification of adenomas per patient. Lastly, and despite the low polyp and adenoma detection rates reported, patients with lower 25(OH)D_3 serum levels in this cohort were, indeed, at higher risk of adenoma detection. Patients considered to be vitamin D deficient (<20 ng/mL) displayed a 20.2% ADR versus only 15.3% for those with "adequate" levels (>30 ng/mL).

The main objective of this analysis was to explore the relationship between colorectal lesions and serum vitamin D. Although the study included a relatively small cohort of patients, a consistent negative association was demonstrated. Remarkably, this association was nonlinear and had a negative logarithmic curve throughout the histological grading of lesions (Fig. 3). A possible explanation for this finding is the effect of non-adenomatous lesions, such as hyperplastic polyps, since previous data show that they do not have a strong association with vitamin D serum levels [7]. This is relevant to understand the role of vitamin D in risk stratification, since it depicts a higher specificity of vitamin D towards adenomatous lesions, which have been associated with a higher risk of cancer.

Two variables – smoking and a positive FHCRC – were found to be independent predictors for detection of any type of neoplastic colorectal lesion. A trend was also towards an association between higher weight, BMI and lower vitamin D serum levels, but these variables failed to reach significance after adjustment in a multivariate model. Again, this may be explained by the contribution of non-adenomatous hyperplastic polyps, for which smoking, for instance, is an established and strong predictor [51,52]. Another interesting finding was that a significant proportion of patients with colorectal lesions reported having their menarche at a younger age. Even though a recently published meta-analysis concluded there was no association between age of menarche and the risk of CRC [53], oestrogens may play a significant role in colorectal carcinogenesis. A post-hoc analysis of the Woman's Health Initiative (WHI) study found that women who did not receive oestrogen therapy benefit from calcium/vitamin D supplementation in terms of CRC risk, and the opposite was true for women in the oestrogen arm [54].

When we further restricted the analysis to adenomatous lesions, vitamin D serum levels was the only variable significantly associated with adenoma detection. After adjusting for cofounding factors, there was a tendency towards a negative correlation with vitamin D, but it failed to reach statistical significance (p = 0.086). Based on the robust evidence supporting this association, we can

Table 2
Analysis of colonoscopy findings. Continuous variables are described by means ± SD. Categorical variables are described by number (%).

Patient characteristics	Colonoscopy findings														
	Polyps					Adenomas					Advanced lesion/adenocarcinoma				
	Polyps (n = 66)	No Polyps (n = 249)	P value	Adjusted OR (95% CI)[a]	P value	Adenoma (n = 46)	No adenoma (n = 269)	P value	Adjusted OR (95% CI)[a]	P value	Advanced lesion (n = 10)	No advanced lesion (n = 305)	P value	Adjusted OR (95% CI)[a]	P value
Age (yr.)	63.8 ± 7.6	62.8 ± 7.0	0.284	–	–	64.7 ± 8.1	62.7 ± 7.0	0.074	–	–	64.6 ± 7.5	62.9 ± 7.1	0.467	–	–
Ethnicity															
White	64 (21.3)	236 (78.7)	0.745	–	–	44 (14.7)	256 (85.3)	1.00	–	–	10 (3.3)	290 (96.7)	1.00	–	–
Non-white	2 (13.3)	13 (86.7)				2 (13.3)	13 (86.7)				0 (0.0)	15 (100)			
BMI (kg/m^2)	29.9 ± 4.6	28.8 ± 4.8	0.080		–	29.9 ± 4.7	28.8 ± 4.7	0.147	–	–	29.7 ± 5.7	29.0 ± 4.7	0.646	–	–
Weight (kg)	**72.6 ± 11.3**	**69.1 ± 12.2**	**0.034***	1.05 (0.99–1.11)	0.066†	72.3 ± 10.7	69.4 ± 12.2	0.643			71.7 ± 12.3	69.8 ± 12.1	0.643		
Smoking, n (%)	11 (16.7)	22 (8.8)	0.065	**3.14 (1.29–7.64)**	**0.012***	4 (8.7)	29 (10.8)	0.799	–	–	1 (10.0)	32 (10.5)	1.00	–	–
FHCRC, n (%)	**11 (16.7)**	**15 (6.0)**	**0.005***	**3.90 (1.48–10.31)**	**0.006***	5 (10.9)	21 (7.8)	0.559	–	–	2 (20.0)	24 (7.9)	0.196	**10.26 (1.19–88.19)**	**0.034***
Vitamin D/calcium therapy, n (%)	41 (65.1)	150 (60.2)	0.678	–	–	30 (69.8)	161 (61.7)	0.310	–	–	8 (80.0)	183 (62.2)	0.332	**9.06 (1.23–64.24)**	**0.027***
Oestrogen therapy, n (%)	36 (57.1)	140 (58.1)	0.892	–	–	24 (55.8)	152 (58.2)	0.766	–	–	7 (70.0)	169 (57.5)	0.528	–	–
Bisphosphonate therapy, n (%)	13 (20.6)	40 (16.6)	0.452	–	–	9 (20.9)	44 (16.9)	0.514	–	–	0 (0.0)	53 (18.0)	0.219	–	–
Age of menarche (yr.)	13.0 ± 1.6	13.3 ± 1.7	0.085†	0.82 (0.67–1.01)	0.065†	13.1 ± 1.8	13.3 ± 1.6	0.411	–	–	13.2 ± 2.0	13.3 ± 1.6	0.833	–	–
Age of menopause (yr.)	50.6 ± 4.8	50.1 ± 4.7	0.879	–	–	50.5 ± 4.5	50.1 ± 4.8	0.642	–	–	50.1 ± 6.5	50.2 ± 4.7	0.846	–	–
Parity (≥1 child), n (%)	62 (93.9)	235 (94.4)	1.00	–	–	43 (93.5)	254 (94.4)	0.734	–	–	9 (90.0)	288 (94.4)	0.450	–	–
25(OH)D_3 (ng/mL)	**23.8 ± 10.7**	**26.0 ± 8.6**	**0.045***	–	–	**22.9 ± 9.6**	**26.0 ± 9.8**	**0.040***	0.96 (0.92–1.00)	0.083†	**18.3 ± 7.4**	**25.8 ± 9.8**	**0.020***	**0.86 (0.77–0.97)**	**0.013***
PTH (pg/mL)	51.4 ± 22.1	49.8 ± 22.3	0.605	–	–	51.3 ± 23.7	49.9 ± 22.0	0.812	–	–	**65.9 ± 26.6**	**49.6 ± 21.9**	**0.044***	–	–
Calcium (mg/dL)	9.7 ± 0.4	9.7 ± 0.4	0.297	–	–	9.7 ± 0.5	9.6 ± 0.4	0.608	–	–	9.7 ± 0.5	9.6 ± 0.4	0.565	–	–
Lumbar spine T-score	−1.0 ± 1.7	−1.2 ± 1.5	0.508	–	–	−1.2 ± 1.7	−1.2 ± 1.5	0.640	–	–	−1.2 ± 1.5	−1.2 ± 1.5	0.790	–	–
Femur T-score	−0.5 ± 0.9	−0.4 ± 3.9	0.460	–	–	−0.7 ± 0.9	−0.4 ± 3.7	0.658	–	–	−0.5 ± 0.6	−0.4 ± 3.5	0.634	–	–

* Values are significant at 0.05 level.
† Values are significant at 0.1 level.
Bold highlights values that are significant at 0.05 level.
[a] Odds Ratio with 95% confidence interval (CI) for a multivariate analysis based on a binary logistic regression model. Model adjusted for: age; body mass index (BMI); weight; smoking status; first degree relatives with CRC (FHCRC); age of menarche; 25(OH)D_3, and PTH serum levels; month of blood collection year and calcium/vitamin D supplementation.

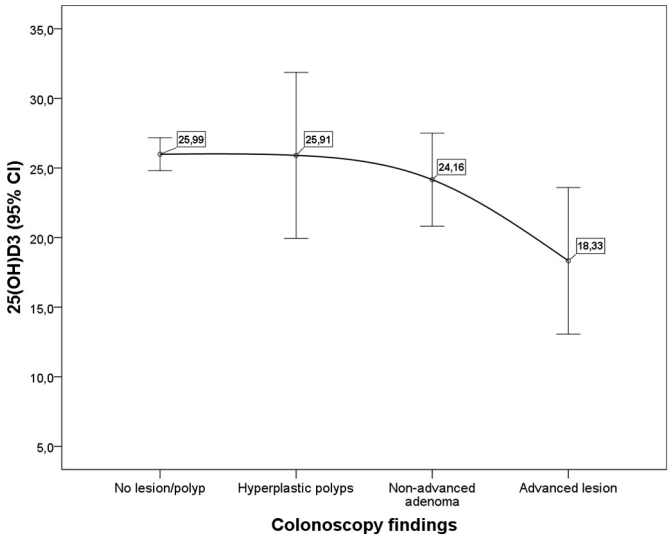

Fig. 3. Serum 25(OH)D_3 levels with 95% Confidence Interval (CI) according to the histological grading of colonoscopy findings. Value units are ng/mL.

Table 3
Analysis of colonoscopy findings according to lesion detection site. Continuous variables are described by means ± SD. Categorical variables are described by number (%).

Patient characteristics	Polyp location		
	Distal (n = 48)	Proximal (n = 13)	P value
Age (yr.)	63.6 ± 6.9	64.5 ± 10.2	0.802
Ethnicity			
Caucasian			
African			
Asian			
BMI (kg/m^2)[a]	30.0 ± 4.7	30.0 ± 4.9	0.936
Smoking, n (%)	10 (20.8)	1 (7.7)	0.429
FHCRC, n (%)	9 (18.8)	1 (7.7)	0.674
Vitamin D/calcium therapy, n (%)	31 (66.0)	9 (69.2)	1.000
Oestrogen therapy, n (%)	27 (57.4)	8 (61.5)	1.000
Bisphosphonate therapy, n (%)	12 (25.5)	1 (7.7)	0.262
Age of menarche (yr.)	12.7 (1.4)	13.5 (1.8)	0.298
Age of menopause (yr.)	50.2 (4.0)	48.8 (5.0)	0.403
Parity (≥1 child), n (%)	45 (93.8)	12 (92.3)	1.000
25(OH)D_3 (ng/mL)	23.2 ± 10.8	24.8 ± 10.1	0.619
PTH (pg/mL)	54.6 (23.3)	45.2 (15.3)	0.177
Calcium (mg/dL)	9.7 (0.5)	9.7 (0.4)	0.916
Lumbar spine T-score	−1.1 (1.7)	−1.0 (1.8)	0.442
Femur T-score	−0.5 (0.9)	−0.4 (1.1)	0.598

speculate that the present analysis might have been underpowered due the relatively small size of its cohort.

Regarding advanced adenomatous lesions, a strong negative association with 25(OH)D_3 serum levels was observed, though the small number of advanced lesions (n = 10) warrants caution when analysing results. The adjusted odds ratio of 0.86 represents an approximately 14% decrease in the risk of advanced lesions for each ng/mL vitamin D increase. Interestingly, calcium/vitamin D supplementation was also independently associated with advanced lesions. This may result from a masking effect, as most of patients were taking supplementation for low vitamin D or correlated comorbidities. It can also be speculated that, even with higher serum vitamin D levels, these patients may have an underlying vitamin D derangement that determines susceptibility to adenomatous colorectal cancer.

Published data suggests that PTH serum levels can be independent predictors of adenoma detection [55]. In this study, although an association with advanced lesion detection was observed in univariate analysis, it was lost after adjusting for confounding factors. It can be hypothesized that higher PTH serum levels only reflect the lower 25(OH)D_3 levels observed in these patients.

Regarding colonic location of the lesions, the published data shows inconsistent results suggesting both no association [34] or, an association with proximal [56] and distal [7] lesions. As so vitamin D and PTH serum levels correlation with polyp colonic location was also explored in our cohort. Due to the small number of polyps in each sublocation, analysis was restricted to proximal versus distal, but a correlation could not be found.

Although this analysis was conducted in a relatively small cohort of peri and post-menopausal women, it supports the previously described negative correlation between vitamin D and adenomatous lesions. Additionally, it proposes that there may be a nonlinear association reflecting a stronger association with higher histological grading.

It is a fact that vitamin D serum levels add relevant information to risk assessment for adenoma detection [17,57,58]. However, the evidence regarding a chemoprotective effect of vitamin D remains an unproven and controversial issue. Although a higher daily vitamin D intake has been associated with a decreased risk of colorectal cancer, especially among women, the two most relevant randomized controlled trials for calcium/vitamin D supplementation failed to achieve their primary endpoint of reducing adenomas and colorectal neoplasia [59,60]. In fact, results from a recently published randomized controlled trial by Crockett et al. suggest that supplementation with calcium, alone or in combination with vitamin D, may increase the risk for serrated polyps [61]. Serrated polyps have a clearly identified carcinogenic process that greatly differs from that of adenomas and therefore this findings should not be translatable to adenoma's risk. Even so, they are certainly a matter of concern when considering the benefits of a chemopreventive strategy. On the other hand, the benefit of any supplementation regimen appears to be dependent on genetic individual factors. Indeed, vitamin D receptor polymorphisms have been shown to directly influence the benefit of vitamin D supplementation in preventing advanced adenomatous lesions [62].

Hence, the most relevant question may be whether, more than a chemoprotective agent, vitamin D may to be a valuable biomarker for risk stratification. In this regard, the development and validation of risk assessment models that take into account biomarkers such as vitamin D could benefit surveillance programs and lead to a better selection of patients in high risk of adenoma recurrence or interval cancer.

Statment of authorship and author contributions

- Collection of data: Pedro Marques da Costa, Inês Martins, Joaquim Neves.
- Statistical analysis: Pedro Marques da Costa.
- Preparation of the manuscript: Pedro Marques da Costa, Inês Martins.
- Critical review of the manuscript: Joaquim Neves, Helena Cortez-Pinto, José Velosa.

Conflicts of interest and funding source

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