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# Colorectal cancer and vitamin D level

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**Abstract:**

This study aimed to evaluate vitamin D levels in patients with CRC. The research was a randomized, double-blind, placebo-controlled vitamin D supplementation experiment in patients undergoing elective CRC surgery. Secondary outcomes including complication incidence, hospital time, post-operative rehabilitation and survival were assessed.

Of the 117 patients assessed for eligibility 58 patients were excluded from the study and 59 patients were randomised. Of the 58 patients excluded, 36 did not meet the inclusion criteria and 22 were excluded for other reasons. These include tumour considered too small for study sample (eight), inadequate time available between notification and surgery (six), study staff unavailable (six) and other logistical reasons (two). In the control arm both tumour and normal tissue samples were collected from 25 of the 30 patients. Of the other five patients, one did not proceed to surgery, the resected tissue of one patient was erroneously placed in formalin in theatre, and the pathologist was not able to provide a tumour sample in the remaining three.

Of the 29 patients recruited to the treatment arm, both tumour and normal tissue samples were collected from 26. The pathologist was unable to provide a tumour sample for three patients. Normal tissue was collected for all but one patient, as staff did not request the normal sample.

This randomized clinical study reported possibly important biological variations between vitamin D and placebo classes. Compared to untreated patients, the fatty acid synthesis and fatty acid beta-oxidation pathways were down-regulated in the patient's tumor tissue. Down-regulation of tumor fatty acid metabolism can delay tumor development. Unmetabolized butyrate (short-chain fatty acid used by colon epithelia) may also precipitate decreased cell proliferation, increased differentiation and apoptosis. Further analysis is needed to clarify the effect of vitamin D on colorectal cancer biology.

**Keywords:** Colorectal, cancer, vitamin D.

## Introduction:

An approximate 14.1 million cancer patients were diagnosed and an estimated 8.2 million cancer deaths occurred globally in 2012, according to Globocan, the World Health Organization's International Cancer Research Agency (IARC) programme (WHO). Worldwide colorectal

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cancer(CRC) is the third most commonly diagnosed malignancy with an estimated 1,360,600 cases diagnosed and an estimated 693,900 deaths in 2012 (IARC 2015).

Incidence rates of CRC are projected to decline in all age groups except in people over 75, decreasing overall by approximately one-quarter in the 45-74 age group. However, the overall burden is projected to continue increasing as a result of population growth and an aging population (MOH 2010).

Vitamin D comprises a group of fat-soluble seco-steroids, similar in structure to steroids. In humans the most important compound in this group is vitamin D3, of which there are several forms (Holick 2006). In contrast, upwards of 90% of an individual's vitamin D requirements can be produced in the skin upon adequate sun exposure (Holick 2000). Nowadays, vitamin D is usually referred to as a pro-hormone given its synthesis in the skin and the diverse array of its activity (Pereira *et al.* 2012).

In 1980, Garland and Garland showed higher mortality rates of colon cancer in regions of the US with low solar radiation, indicating that this finding could be related to lack of skin synthesis of vitamin D (Garland and Garland 1980). Furthermore, in urban countries, colon cancer deaths were higher than in rural areas. In the US, the same authors documented an inverse correlation between vitamin D and CRC (Garland et al. 1989).

Rectal cancer had a greater correlation. Study of 1822 colon and 868 rectal cancers indicated that elevated circulating calcidiol levels were correlated with major CRC reduction (OR=0.66, 95 percent CI=0.54-0.81 for upper versus lower categories). The reverse correlation was better for rectal cancer (OR=0.5, CI=0.28-0.88 for upper and lower categories) (Lee *et al.* 2011).

This study aimed to evaluate vitamin D levels in patients with CRC.

**Materials and methods:**

Patients with a pre-operative diagnosis of CRC, confirmed by colonoscopy or computerized tomography colonography (CTC), and scheduled to undergo elective surgery. Patients with acute presentations, such as bowel obstruction or perforation, were not eligible due to the

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requirement for a minimum of seven days between randomization and administration of study drug, and surgery.

### *Inclusion criteria*

Patients were included in the study if all of the following were applicable:
• Patients with CRC, confirmed by colonoscopy or CTC, and scheduled for elective surgery.
•Male or female, aged 18 years or over.

• Patient serum calcium level <2.6 mmol/L, and serum Vit. D level <125 nmol/L.

### *Exclusion criteria*

Patients were excluded from the study if any of the following were applicable:
•Aged less than 18 years of age.

• Concomitant use of prescription strength cholecalciferol.

•Participation in research studies in the previous 12 weeks involving investigational product.

•Pregnant or lactating.

•Hypercalcaemia (>2.6 mmol/L).

•Hyperparathyroidism.
•History of kidney stones.

•Renal failure (requiring renal replacement therapy).

• Advanced liver disease (Childs-Pugh B or C).

• Any other significant disease or disorder which, in the opinion of the Investigator, may either put the patient at risk because of participation in the study, or may influence the result of the study or the participant's ability to participate in the study.

### *Baseline measurements*

#### 1.*Demographic*

Demographic information including age, gender, and season of diagnosis was collected and entered into the database.

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**2.*Clinical***

Clinical data were collected from patient clinical notes and entered prospectively into the database. This included date, height and weight (used to calculate body mass index= BMI), co-morbidities, the American Society of Anaesthesiology score, and site of tumour.The American Society of Anaesthesiology (ASA) score is the standard method used to classify peri-operative risk and is determined by patient co-morbidities (Dripps *et al.*1961).

***3.Laboratory***

Blood samples were collected at baseline and again immediately prior to surgery for serum calcium and vitamin D levels. These were measured by using Biolabo Kits, these were done according to manufacturer instructions.

**Intervention**

Patients were randomised to a single dose of 200,000 international units (IU), equivalent to 5 mg of vitamin D in the form of cholecalciferol, or an identical placebo. They were instructed to take the dose between seven and 21 days prior to the scheduled surgery date. The study treatment was self-administered and the date of study drug ingestion was recorded.

***Specimen collection and storage***

Resection specimens were collected fresh from the operating theatre and taken to the pathology laboratory. The attendant pathologist dissected the specimen and removed normal and tumour tissue samples for the study. Tumour tissue samples were taken from within the invasive margin, avoiding the necrotic regions of the tumour, with the aim of collecting non-necrotic representative tumour tissue. Normal tissue was taken from at least 5 cm from the tumour to reduce the likelihood of contamination with tumour cells.

**Results:**

Of the 117 patients assessed for eligibility 58 patients were excluded from the study and 59 patients were randomised. Of the 58 patients excluded, 36 did not meet the inclusion criteria and 22 were excluded for other reasons. These include tumour considered too small for study sample (eight), inadequate time available between notification and

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In the control arm both tumour and normal tissue samples were collected from 25 of the 30 patients. Of the other five patients, one did not proceed to surgery, the resected tissue of one patient was erroneously placed in formalin in theatre, and the pathologist was not able to provide a tumour sample in the remaining three.

Of the 29 patients recruited to the treatment arm, both tumour and normal tissue samples were collected from 26. The pathologist was unable to provide a tumour sample for three patients. Normal tissue was collected for all but one patient, as staff did not request the normal sample (Table 1).

Table 1. Baseline patient characteristics.

| **Characteristic** | **Placebo Group** (n=30) | **Vit D Group** (n=29) | **Total** (n=59) | **P Value** |
|---|---|---|---|---|
| **Age (years), median (range)** | 70(46-84) | 71(38-86) | 70(38-86) | 0.98 |
| **Sex, n(%)** | | | | 0.44 |
| Men | 14(47) | 17(59) | 31(53) | |
| Women | 16(53) | 12(41) | 28(47) | |
| Season of diagnosis, n (%) | | | | 0.7 |
| Winter | 5(17) | 8(28) | 13(22) | |
| Spring | 8(27) | 5(17) | 13(22) | |
| Summer | 6(20) | 7(24) | 13(22) | |
| Autumn | 11(37) | 9(31) | 20(34) | |

***Clinical***

Baseline patient clinical characteristics of BMI, ASA score and tumour site were well matched between the two arms of the study (Table 2)

| **Characteristic** | **Placebo Group** (n=30) | **Vit D Group** (n=29) | **Total** (n=59) | **P Value** |
|---|---|---|---|---|
| **BMI (kg/m2),mean (sd)** | 29.2(4.1) | 28.0(4.7) | 28.6(4.4) | 0.31 |
| **ASA group, n(%)** | | | | 0.38 |
| I | 6(20) | 5(17) | 11(18) | |

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| | | | | |
|---|---|---|---|---|
| II | 21(70) | 18(62) | 39(66) | |
| III | 2(7) | 6(21) | 9(15) | |
| IV | 1(3) | 0(0) | 1(2) | |
| **Tumour site, n(%)** | | | | 0.32 |
| Right colon | 16(53) | 12(41) | 28(47) | |
| Left colon | 7(23) | 5(17) | 12(20) | |
| Rectum | 7(23) | 12(41) | 19(32) | |

## Laboratory

Baseline serum calcidiol levels were measured for all of the 59 randomised participants, and were similar between the two study groups (Table 3). The baseline levels were higher in patients recruited to the study during summer and autumn.One patient had a baseline calcidiol level of 104 nmol/L. This patient was taking cod liver oil, which is known to contain high levels of vitamin D. The patient is included in the intent-to-treat analysis. All participants had serum calcium levels within the normal range. Baseline calcidiol levels were significantly higher in patients who were diagnosed in summer and autumn (Table 3).

**Table 3. Baseline serum levels.**

| **Characteristic** | **Placebo Group** (n=30) | **Vit D Group** (n=29) | **Total** (n=59) | **P Value** |
|---|---|---|---|---|
| **Baseline calcidiol (nmol/L), mean (sd)** | 58(27) | 58(23) | 58(25) | 0.96 |
| **Baseline calcium (mmol/L), mean (sd)** | 2.26(0.13) | 2.29(0.13) | 2.27(0.13) | 0.39 |
| **Baseline calcidiol (nmol/L)/Season of diagnosis** | | | | * <0.001# |
| Winter | | | 45(23) | |
| Spring | | | 45(26) | |
| Summer | | | 73(14) | |
| Autumn | | | 64(24) | |

## *Clinico-pathological*

Baseline clinico-pathological characteristics of tumour stage and grade are shown in Table 4. These are similar in the treatment and control arms.

**Table4. Baseline patient characteristics.**

| | **Placebo Group** (n=30) | **Vitamin D Group** (n=29) | **Total** (n=59) | **P Value** |
|---|---|---|---|---|

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| Characteristic | | | | |
|---|---|---|---|---|
| **Tumour stage TNM, n(%) ( n=59)** | | | | 0.86 |
| I | 5(17) | 7(24) | 12(21) | |
| II | 11(37) | 11(38) | 22(38) | |
| III | 11(37) | 9(31) | 20(34) | |
| IV | 3(10) | 2(7) | 5(8) | |
| **Tumour grade,n(%)** | | | | 1 |
| G1 | 0(0) | 0(0) | 0(0) | |
| G2 | 24(83) | 24(83) | 48(81) | |
| G3 | 5(17) | 5(17) | 10(17) | |
| Missing* | 1(3) | 0(0) | 1(2) | |

## *Secondary outcomes*

## *Vitamin D and calcium levels*

Post treatment calcidiol and calcium levels (levels determined from pre-incision bloods) were measured again immediately prior to surgery in all 58 patients who went forward for surgery (Table 5). Pairwise comparisons were performed to examine serum vitamin D levels before and after study drug. Patients randomised to vitamin D had a significant increase in vitamin D levels above their baseline levels. Post treatment calcium levels remained below 2.6 mmol/L in both groups.

**Table 5. Secondary outcomes: Vitamin D levels.**

| | Placebo Group (n=29) | Vitamin D Group (n=29) | Total (n=58)* | P Value |
|---|---|---|---|---|
| **Postdose calcidiol (nmol/L),mean (sd)** | 49(19) | 87(22) | 69(28) | <0.001# |
| **Pre & post calcidiol comparison** | 0.2 | <0.001$ | 0.036 | |
| **Postdose calcium (mmol/L),mean (sd)** | 2.26(0.09) | 2.25(0.12) | 2.25(0.10) | 0.61 |
| **Pre & post calcium comparison** | 0.97 | 0.19 | 0.35 | |

## Discussion:

Serum calcidiol concentrations were found to be significantly higher in patients recruited during summer and autumn. Vitamin D synthesis in the skin varies depending on the season. This phenomenon has been described (Holick 1995).

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Research carried out in New Zealanders by Rockell et al. (2006) found that mean calcidiol concentrations varied markedly with season with the greatest difference observed between summer and spring levels. Similar findings were reported by other New Zealand researchers, Livesey et al. (2007) and Bolland et al. (2006).

Studies conducted in experimental animals support both protective and therapeutic effects of vitamin D in CRC. Delivering a western style diet (high in fat, low in vitamin D and calcium) to wild-type mice induced colonic crypt hyperplasia and colon dysplasia. The number of pre-neoplastic lesions was elevated in intestinal carcinogenesis mouse models. Supplementation with vitamin D and calcium suppressed these effects in the mice (reviewed in (Lamprecht and Lipkin 2003) and (Ordonez-Moran *et al.* 2005).Human CRC xenografts, established by implanting CRC cell lines subcutaneously into immuno-suppressed mice, are frequently utilised in pre-clinical anti-cancer drug development. Several studies have demonstrated the inhibitory nature of calcitriol towards the growth of colorectal xenografts (reviewed in (Ordonez-Moran *et al.* 2005),(Deeb *et al.* 2007) and (Kang *et al.* 2011). Numbers of colorectal tumours induced in mice and rats by various chemical carcinogens were also decreased by the administration of calcitriol (reviewed in (Ordonez-Moran *et al.* 2005).The *APC* min/+ mouse is a model of intestinal tumouri-genesis that carries a mutated allele of *APC* and spontaneously grows numerous neoplasias throughout the intestinal tract (Su *et al.* 1992). Administration of calcitriol decreases tumour load in *APC* min/+mice (Huerta *et al.* 2002). These findings have been confirmed more recently and the researchers also demonstrated that calcitriol administration reduces nuclear β-catenin levels, down-regulates the expression of *MYC* (encodes a transcription factor with roles in cell cycle progression and apoptosis) and elevates that of E-cadherin (epithelial cadherin, a cell-cell adhesion glycoprotein) in the colon of *APC* min/+ mice (Xu *et al.* 2010).

The integration and overall analysis of relevant epidemiological studies is limited. Manystudies do not account for endogenous vitamin D synthesis from UV exposure and are restricted by measurement error of dietary vitamin D intake. Such shortcomings can be overcome by measuring circulating calcidiol concentration, which is a useful biomarker

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for providing an overall estimate of vitamin D status accounting for both UV exposure and dietary intake (Pereira *et al.* 2012).

A prospective, observational study, demonstrated that in patients with CRC, elevated pre-diagnosis calcidiol levels were correlated with a significant improvement in overall survival (Ng *et al.* 2008).

Recently, Giovannucci rereviewed the epidemiology of CRC and vitamin D, commenting that despite employing numerous approaches to quantify vitamin D for diverse endpoints and in diverse populations the inverse association was highly consistent and therefore strongly indicative of a causal relationship (Giovannucci 2013).

## Conclusion:

CRC occurred mostly in patients with low vitamin D.

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