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Independent and joint effects of serum 25-hydroxyvitamin D and calcium on ovarian cancer risk: A prospective nested case–control study

Adetunji T. Toriola [a,b,*], *Helja-Marja Surcel* [a], *Agborsangaya Calypse* [a,b], *Kjell Grankvist* [c], *Tapio Luostarinen* [d], *Annekatrin Lukanova* [e], *Eero Pukkala* [b,d], *Matti Lehtinen* [a,b]

[a] *National Institute for Health and Welfare, Finland*
[b] *Tampere School of Public Health, University of Tampere, Finland*
[c] *Department of Medical Biosciences, Umeå University, Umeå, Sweden*
[d] *Finnish Cancer Registry, Institute for Statistical and Epidemiological Cancer Research, Helsinki, Finland*
[e] *Department of Cancer Epidemiology, German Cancer Research Centre, Heidelberg, Germany*

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ABSTRACT

Introduction: Ovarian cancer has very few known modifiable risk factors but dietary studies suggest a role for vitamin D and calcium in the prevention of ovarian cancer. Thus, we investigated the association between pre-diagnostic serum calcium and 25-hydroxyvitamin D (25-OHD) on the risk of later development of ovarian cancer.

Methods: We conducted a population-based nested case–control study within the Finnish Maternity Cohort (FMC). The cohort subset comprised 172 ovarian cancer cases with 172 matched controls (age ± 1 year, parity and season of blood donation ± 2 weeks).

Results: We observed a significant inverse association between calcium and ovarian cancer risk. Relative risk (estimated as odds ratio, OR) comparing the highest quartile to the lowest quartile was significantly decreased; 0.41 [95% confidence interval (CI) 0.19–0.85, *P*-trend 0.004]. Even though a comparable association between 25-OHD and ovarian cancer did not reach statistical significance (OR 0.57, 95% CI 0.26–1.24, *P*-trend 0.07), having sufficient (>75 nmol/L) serum 25-OHD levels compared to insufficient serum 25-OHD was associated with a significantly decreased risk of ovarian cancer (OR 0.32; 95% CI 0.12–0.91, *p*-value 0.03). No synergistic protective interaction between high levels of calcium and 25-OHD against ovarian cancer was observed, the joint effect being just multiplicative.

Conclusion: Calcium and vitamin D act independently to reduce the risk of ovarian cancer. The decreased risk of ovarian cancer associated with pre-diagnostic serum calcium and vitamin D needs to be evaluated further for possible new insights into ovarian cancer prevention.

* *Corresponding author*: Address: National Institute for Health and Welfare, PL 310, FIN-90101, Finland. Tel.: +358 20610 6210; fax: +358 20610 6251.
E-mail addresses: Adetunji.toriola@uta.fi, Adetunji.toriola@thl.fi (A.T. Toriola).
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1. Introduction

Ecological, dietary and experimental studies suggest that vitamin D may be associated with reduced risk of ovarian cancer.[1–4] So far, only three epidemiological studies have directly explored this association by relating serum 25-hydroxyvitamin D (25-OHD) to risk, but the results were not conclusive.[5–7] Nevertheless, in two of the three studies, women with sufficient vitamin D concentrations tended to have lower risk of ovarian cancer overall.[5,6] Thus, more studies to characterise a possible association of ovarian cancer with vitamin D and explore the effect of additional factors that may modify risk are of interest.

The classical function of vitamin D is to facilitate the intestinal absorption of calcium but evidence is mounting to suggest that the actions of vitamin D and calcium on physiologic processes may be synergistic.[8,9] High extracellular calcium can modulate vitamin D metabolism in favour of increased conversion to 1,25-dihydroxyvitamin D (the active form of vitamin D) which in turn may up-regulate the expression of the calcium receptor and increase intestinal calcium absorption.[9] In support, a randomised clinical trial recently demonstrated that while calcium supplementation reduced the risk of all-cause cancer among women, the protective effect was more pronounced among women with combined calcium and vitamin D supplementation.[10] Along the same lines, the protective effect of high dietary calcium intake on colorectal cancer incidence was strongest in subjects with highest vitamin D intake in a large pooled analysis of 10 cohort studies.[11]

The independent role of serum calcium in ovarian cancer has not been explored so far. While some studies have observed an inverse association of ovarian cancer with dietary intake of calcium, others have observed positive or no associations.[12–17] Nevertheless, the relationship between serum calcium concentrations and dietary calcium intake is complex. Serum calcium concentrations are tightly regulated and there is very little correlation between it and dietary calcium intake.[18] To the best of our knowledge, no previous study has investigated the effect of serum calcium and the joint effects of serum calcium and vitamin D levels on ovarian cancer risk. The aim of this study was to evaluate possible independent and joint effects of vitamin D and calcium on the risk of ovarian cancer.

2. Material and methods

2.1. Finnish Maternity Cohort

This study is a prospective, population based case–control study nested within the Finnish Maternity Cohort (FMC). The FMC was established by the National Institute for Health and Welfare (formerly National Public Health Institute), Finland, in 1983.[19] Following an informed consent, first trimester blood samples are withdrawn from pregnant women at the municipal maternity care units to screen for intrauterine infections. After the screening has been done, the remaining sample (1–3 mL of serum) is stored in polypropylene cryo vials at −25 °C in a well-protected biorepository at the National Institute for Health and Welfare, Oulu. More than 98% of pregnant women in Finland have donated blood samples to the cohort since 1983 and currently about 1.6 million samples are kept in storage. Each year about 60,000 new serum samples are added to the repository.

2.2. Identification of cases and controls

Incident ovarian cancer cases were identified by the population-based Finnish Cancer Registry (FCR). All cancer cases diagnosed in Finland since 1953 are reported to the FCR (reporting mandatory since 1961). The coverage of the FCR is virtually complete with no losses to follow-up.[20] Every resident of Finland has a unique personal identity code that is also used in official health registries like the FMC and the FCR. These codes were used as the linkage key. Ovarian cancer cases who had donated serum samples to the FMC more than once, at least 1 year apart, before cancer diagnosis were selected for this study. Of the 215 cases that fulfilled these criteria, those who donated their last sample within 1 year before the cancer diagnosis and those who had been selected for a previous study of ovarian cancer and vitamin D within this cohort[5] were excluded, leaving 172 cases. There were 166 cases with histological confirmation of which 68 (41%), 60 (36%) and 15 (9%) were serous, mucinous and endometroid cancers, respectively.

Eligible controls were women from the FMC who were alive and free of cancer at the time of diagnosis of the index case and who also had donated at least two serum samples during different pregnancies to the FMC. The controls were matched for (i) age at sample withdrawal ± 1 year, (ii) parity and (iii) date of index blood sampling ±2 weeks for both sets of samples. One control with paired samples was selected for every case. For this study, the first set of serum samples for cases and controls was used.

The study was approved by the ethical committee of the National Institute for Health and Welfare, Finland.

2.3. Laboratory analysis

Quantification of 25-OHD was performed at the Department of Medical Biosciences, University of Umeå, Umeå, Sweden, using a 25-OHD radioimmunoassay (RIA) from IDS Ltd., Boldon, United Kingdom. The manufacturer stated a specificity (% cross-reactivity) of 100% for 25-OHD3, 75% for 25-OHD2, 100% for 24, 25-OH2D3 and less than 0.01% or 0.3% for cholecalciferol (D3) and ergocalciferol (D2), respectively. The within, between and total coefficients of variation (CV) of the assay were 4.3%, 17.1% and 17.7% at level 32.9 nmol/L, respectively. Serum 25-OHD concentrations were defined as follows: (i) sufficient > 75 nmol/L, (ii) relative insufficient 50–75 nmol/L and (iii) insufficient or deficient <50 nmol/L.[21] Outliers were defined as concentrations exceeding three times the interquartile range and were set to missing (n = 1 for 25-OHD).

Quantification of serum calcium was performed at the Clinical Chemistry Laboratory, Östersunds Hospital, Östersund, Sweden, using the Roche/Hitachi cobas c system analyzer (Roche Diagnostics GmbH, D68298 Mannheim, Germany). The total coefficient of variation (CV) of the assay was 2.4% at level 2.0 mmol/L. Case and control samples belonging to the same study set were assayed together, but

ordered randomly and labelled to mask case–control status. Three samples had insufficient volume to perform the laboratory assays.

2.4. *Statistical analysis*

Descriptive statistics are presented as mean (standard deviation) for all other data and median and percentiles for calcium and 25-OHD.

Quartile cut-off points for both 25-OHD and calcium were determined using the distribution among the controls. Conditional logistic regression was used to calculate odds ratio with 95% confidence interval (OR with 95% CI) for ovarian cancer in the different quartiles of 25-OHD and calcium using the lowest quartiles as the reference category. Tests for trend were calculated using continuous scale of the variables, log-transformed for 25-OHD because the overall distribution was slightly skewed even though the season-specific distributions were normal. The effect of adjustment for age at first full-term pregnancy, age at last full-term pregnancy, gestational day at blood donation and region of residence (North, South, West, East and Central Finland) were investigated in multivariate models, and variables that altered risk estimates by more than 5% were retained in the final models (age at first full-term pregnancy and region of residence). Mutual adjustments of vitamin D models for calcium concentrations and vice versa were also performed but the results are very identical to those obtained without the mutual adjustments and are thus not presented. Secondary analyses were carried out excluding cases (and their matched controls) whose cancers were diagnosed within 1–3 years of serum sampling (analysis was also done excluding cases diagnosed within 1–2 years of sampling but the results were identical to those obtained excluding cases diagnosed within 1–3 years and thus not presented) and for women with sufficient/insufficient serum vitamin D (>75 nmol/L).[21]

To determine the possible synergistic effects of 25-OHD and calcium, we categorised the subjects into four groups based on each individual's combined 25-OHD and calcium status. (i) Low 25-OHD /low calcium – women with both 25-OHD and calcium categories within the 1st 3 quartiles, i.e. (25-OHD Q1, Q2, Q3 + calcium Q1, Q2, Q3). This represented the reference category, (ii) high 25-OHD/low calcium – women whose 25-OHD were within the 4th quartile but whose calcium were within the 1st 3 quartiles (25-OHD Q4 + calcium Q1, Q2, Q3), (iii) low 25-OHD/high calcium – women with 25-OHD within the 1st 3 quartiles but calcium within the 4th quartile (25-OHD Q1, Q2, Q3 + calcium Q4) and (iv) high 25-OHD/high calcium – both 25-OHD and calcium were within the 4th quartiles (25-OHD Q4 + calcium Q4). Testing for effect modification was done with a likelihood ratio test to compare two nested models, by considering the difference between the model-specific scaled deviances. All statistical analyses were performed using SPSS 18 for windows (SPSS, Inc., Chicago IL). Two sided $p < 0.05$ was considered statistically significant.

3. Results

Mean age at serum sampling for both cases and controls was the same (29.9 years). Likewise ages at first full-term and last full-term pregnancies and number of pregnancies were almost identical for both cases and controls. The mean lag time between serum sampling and cancer diagnosis was 6.4 years (range 1–13.5 years). Median 25-OHD concentrations were 39.2 nmol/L (90th percentile 65.4) and 40.0 nmol/L (90th percentile 73.4); while median calcium concentrations were 2.3 mmol/L and 2.4 mmol/L for cases and controls, respectively (Table 1). There was no correlation between serum 25-OHD and calcium concentrations among cases (rs = −0.02, *p*-value 0.82) and controls (rs = 0.07, *p*-value 0.39).

Mean serum 25-OHD concentrations for both cases and controls were highest in summer and autumn and lowest in winter. Though the season-matched mean serum 25-OHD levels were higher among controls compared to cases for all the seasons, the differences in means were not statistically significant. The highest differences in means were observed for spring (8.5 nmol/L) and were the lowest for summer (3.9 nmol/L) (Fig. 1).

Odds ratio comparing the fourth quartile of serum 25-OHD concentration to the first quartile was 0.63 (95% confidence interval (CI) 0.29–1.34, *P*-trend 0.10). Adjusting for age at first full-term pregnancy and region of residence, the odds ratio was OR 0.57 (95% CI 0.26–1.24, *P*-trend 0.07). When cases whose cancers were diagnosed within 1–3 years of serum sampling were excluded from the analysis, the odds ratio was slightly reduced and the trend test reached statistical significance, OR 0.43 (95% CI 0.18–1.05, *P*-trend 0.02) (Table 2). Women with sufficient 25-OHD concentrations (>75 nmol/L) had a significantly reduced risk of ovarian cancer compared to those with insufficient serum concentrations (OR 0.32; 95% CI 0.12–0.91, *p*-value 0.03). The number of women with sufficient serum 25-OHD concentrations were, however, small (20 women, 5 cases and 15 controls). The results were identical when season-defined quartiles were used.

Increasing serum calcium concentration was also inversely associated with ovarian cancer risk. Comparing the highest to the lowest quartile, the odds ratios were 0.46 (95% CI 0.23–0.95, *P*-trend 0.005) and 0.41 (95% CI 0.19–0.85, *P*-trend 0.004) in the first and multivariate adjusted models, respectively. Exclusion of cases diagnosed within 1–3 years of serum sampling had no material effect on the point estimate; OR 0.37 (95% CI 0.16-0.85, *P*-trend 0.002) (Table 3).

Compared to women who had low calcium/low 25-OHD concentrations, those who had high calcium/high 25-OHD levels had an odds ratio of 0.26 (95% CI 0.07–0.90). Likewise, the odds ratios among women with high calcium/low 25-OHD and low calcium/high 25-OHD were OR 0.41 (95% CI 0.19–0.87) and 0.51 (95% CI 0.29–1.05), respectively (see Table 4). Calcium does not modify the effect of 25-OHD ($p = 0.25$) and 25-OHD does not modify the effect of calcium ($p = 0.12$). The interaction is multiplicative.

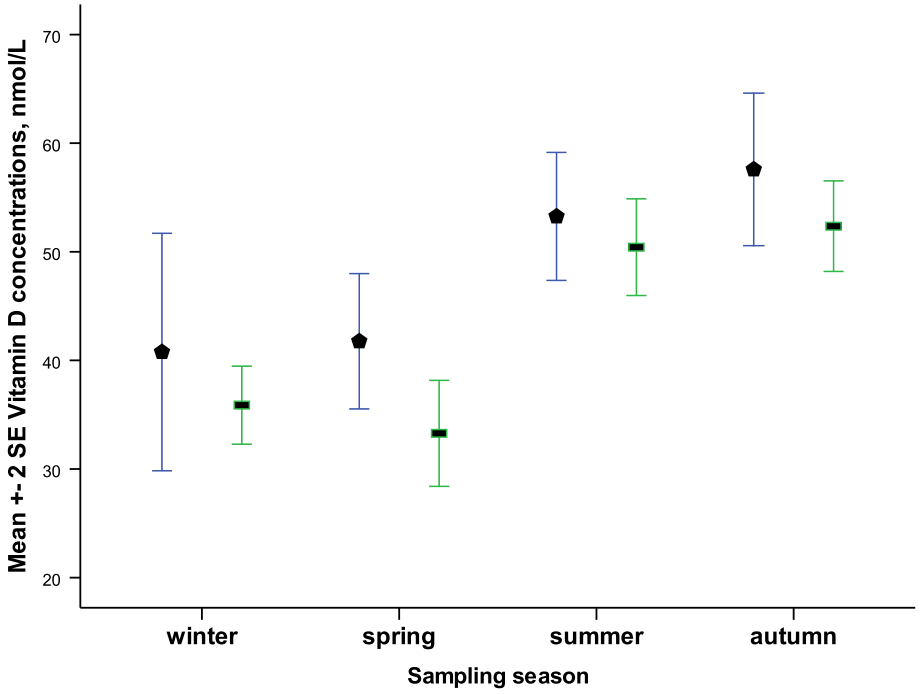
4. Discussion

To the best of our knowledge, this is the first prospective study to examine the association of both serum calcium and 25-OHD with the risk of ovarian cancer. Our findings (i)

Table 1 – Baseline characteristics of ovarian cancer cases and matched, incidence density sampled controls who donated serum samples to the Finnish Maternity Cohort between 1983 and 2007.

	Cases (n = 172)	Controls (n = 172)
Age at serum sampling, years	29.9 (4.3)	29.9 (4.3)
Age at first full-term pregnancy, years	26.9 (4.3)	27.4 (4.3)
Age at last full-term pregnancy, years	31.4 (4.1)	32.1 (4.6)
Age at cancer diagnosis, years	36.4 (5.1)	
Lag time to cancer diagnosis, years (minimum, maximum)	6.4 (1, 13.5)	
Number of pregnancies	2.5 (1.0)	2.5 (1.0)
Gestational day at blood donation, d	76.6 (21.3)	78.9 (27.7)
25-OHD concentration nmol/L, median (10th, 90th percentile)	39.2 (23.8, 65.4)	40.0 (24.6, 73.4)
Calcium concentration mmol/L, median (10th, 90th percentile)	2.3 (1.9, 2.6)	2.4 (2.1, 2.8)

All values are expressed as mean (standard deviation) unless otherwise stated.



Error bars for cases are on the right while those for controls are on the left

Fig. 1 – Mean serum vitamin D concentration (±2 SE) by season of blood donation among cases and controls.

Table 2 – Relative risk (estimated as odds ratio, OR, with 95% confidence intervals, CI) of ovarian cancer by quartile of serum 25-hydroxyvitamin D concentrations among Finnish women followed up to 13 years after sample donation.

	Quartile 1	Quartile 2	Quartile 3	Quartile 4	P-trend	Q4 versus Q1–3
Quartile values, nmol/L	<31.5	31.5–40.0	40.1–57.7	⩾57.8		
n, cases/control[a]	42/43	44/44	52/42	30/43		
OR	1.0 (reference)	1.05 (0.58–1.92)	1.25 (0.67–2.32)	0.63 (0.29–1.34)	0.10	0.56 (0.30–1.04)
OR, adjusted[b]	1.0 (reference)	1.01 (0.54–1.87)	1.13 (0.60–2.12)	0.57 (0.26–1.24)	0.07	0.54 (0.28–1.02)
Excluding cases who donated serum samples within 1–3 years of cancer diagnosis						
Quartile values	<32.0	32.1–40.5	40.6-58.1	⩾58.2		
n, cases/control	36/33	37/33	35/35	25/36		
OR, adjusted[b]	1.0 (reference)	0.93 (0.48–1.82)	0.79 (0.39–1.63)	0.43 (0.18–1.05)	0.02	0.49 (0.24–1.01)

[a] Controls were matched for age at serum sampling, parity and date of blood donation (±2 weeks).
[b] Adjusted for age at first full-term pregnancy and region of residence.

Table 3 – Relative risk (estimated as odds ratio, OR, with 95% confidence intervals, CI) of ovarian cancer by quartile of serum calcium concentrations among Finnish women followed up to 13 years after sample donation.

	Quartile 1	Quartile 2	Quartile 3	Quartile 4	P-trend
Quartile values, mmol/L	<2.2	2.2–2.4	2.4–2.6	⩾2.6	
n, cases/control[a]	46/39	50/45	48/44	26/44	
OR	1.0 (reference)	0.99 (0.54–1.85)	0.86 (0.46–1.60)	0.46 (0.23–0.95)	0.005
OR, adjusted[b]	1.0 (reference)	1.04 (0.55–1.96)	0.84 (0.44–1.61)	0.41 (0.19–0.85)	0.004
Excluding cases who donated serum samples within 3 years of cancer diagnosis					
Quartile values	<2.2	2.2–2.4	2.4–2.6		
n, cases/control	35/31	43/35	37/32	20/39	
OR, adjusted[b]	1.0 (reference)	1.26 (0.59–2.67)	0.97 (0.46–2.08)	0.37 (0.16–0.85)	0.002

[a] Controls were matched for age at serum sampling, parity and date of blood donation (±2 weeks).
[b] Adjusted for age at first full-term pregnancy and region of residence.

Table 4 – Joint effect of exposure of 25-hydroxyvitamin D and calcium on the relative risk (estimated as odds ratio, OR, with 95% confidence intervals, CI) of ovarian cancer among Finnish women.

	Group 1	Group 2	Group 3	Group 4
Case/control	116/96	26/32	21/33	5/11
OR, adjusted[a]	1.0 (reference)	0.51 (0.29–1.05)	0.41(0.19–0.87)	0.26 (0.07–0.90)

Low vitamin D/low calcium – Vit D (Q1, Q2, Q3) and calcium (Q1, Q2, Q3). High vitamin D/low calcium – Vit D (Q4) and calcium (Q1, Q2, Q3). Low vitamin D/high calcium – Vit D (Q1, Q2, Q3) and calcium (Q4). High vitamin D/high calcium– Vit D (Q4) + calcium (Q4).
[a] Adjusted for age at first full-term pregnancy and region of residence.

a strong inverse association between serum calcium concentration and ovarian cancer risk with and without high serum 25-OHD concentrations, (ii) a borderline inverse association between high serum 25-OHD concentrations and ovarian cancer risk, but (iii) no synergistic interaction between 25-OHD and calcium with regard to ovarian cancer risk.

4.1. *Calcium and ovarian cancer*

An inverse relationship between dietary calcium and ovarian cancer has been reported in some studies but not all.[12–17] However, serum calcium does not reflect dietary calcium intake as this relationship is influenced by vitamin D, parathyroid hormone (PTH) and other dietary factors.[18,22] The Norwegian study[18] observed no significant effect of dietary intake of calcium and vitamin D on serum calcium concentrations in women. Though the biological processes by which calcium may influence ovarian cancer are largely unknown, possible mechanisms include (i) the effects of calcium on apoptosis, cell growth and proliferation,[23,24] (ii) effects of the calcium receptor (CaR) on cell proliferation and differentiation[24,25] and (iii) effects of calcium on down-regulating PTH production.[26]

Calcium regulates many important steps in the apoptotic pathway from early signalling to chromatin cleavage but the precise molecular mechanisms involved are not clear.[23] The calcium receptor also regulates homeostasis in response to changes in extracellular calcium concentrations. It modulates the equilibrium between proliferation and differentiation in response to changes in extracellular calcium concentrations.[24,25] Loss of CaR-induced response to extracellular calcium has been observed in ovarian cancers.[25]

In hypocalcaemia, PTH stimulates the conversion of 25-hydroxyvitamin D to 1,25-dihydroxyvitamin D in the kidneys. This leads to an increase in vitamin D availability which subsequently leads to increased calcium absorption and serum calcium.[26] The increase in serum calcium then provides a negative feedback causing a suppression of PTH production and reducing serum PTH levels. It has been hypothesised that PTH may be a tumour promoter acting as a co-mitogen and anti-apoptotic factor.[26] It also increases hepatic and osteoblastic synthesis of insulin-like growth factor-1 (IGF-1)[26,27] which has strong mitogenic effects and has been implicated in the pathogenesis of ovarian and other cancers.[28,29] The presence of growth regulating PTH receptors and PTH-related protein (PTH-rP) has been noted in cancer cells, including ovarian cancer.[30] However, to what extent the PTH-induced increases in IGF-1 concentrations alter the physiological balance of IGF-1 is unknown. Hence, by down-regulating PTH production, calcium potentially mitigates against the mitogenic and anti-apoptotic effects of PTH. This same hypothesis has also been proposed as one of the likely means by which vitamin D may protect against cancer,[26] but in our study, the protective effect of high calcium concentrations was seen irrespective of the vitamin D status.

4.2. *Vitamin D and ovarian cancer*

No previous study has observed a significant overall association between serum vitamin D and risk of ovarian cancer but borderline inverse associations have been reported in subgroup analyses.[5,6] We also observed a borderline inverse relationship between serum vitamin D and ovarian cancer in a secondary analysis excluding cases who donated samples

within 3 years prior to cancer diagnosis for which the trend test was statistically significant. However, there have been no previous reports of significant inverse association among women with sufficient serum vitamin D concentrations, a relative rarity in Finland, despite dietary fortification programmes since 1994. Previous studies by Tworoger et al. and us have observed borderline inverse associations among such groups.[5,6]

The present results differ slightly when compared to our earlier study within this cohort.[5] In that study, we conjectured that one of the reasons why no association was apparent could be because of the low serum 25-OHD within that cohort subset. This may hold true because the median serum 25-OHD among cases and controls in the present study is about 5 nmol/L higher than that of our earlier study. It also suggests that a minimum level of serum vitamin D is necessary to offer protection against ovarian cancer and in population groups where serum vitamin D concentrations are low; such protective effects may not be evident.

A recent large study observed a significant positive relationship between the number of FOK1 f alleles and ovarian cancer risk suggesting that the vitamin D pathway indeed plays a role in ovarian carcinogenesis,[31] but perhaps not in direct conjunction with calcium metabolism. Studies on the association between vitamin D receptor polymorphism and the risk of ovarian cancer have yielded conflicting results[31–33], indicating that the modes of action of vitamin D in the ovarian cancer context remain open.

4.3. *Joint effects of vitamin D and calcium in ovarian cancer*

We observed no evidence of a synergistic interaction between serum vitamin D and calcium on ovarian cancer risk. While calcium was independently associated with a reduced risk of ovarian cancer irrespective of vitamin D levels, vitamin D was independently associated with a non-significantly reduced risk of ovarian cancer. This suggests that the protective effects observed among women with high calcium and high vitamin D levels were likely to be mediated by the effects of high calcium levels. The concept of a possible synergistic effect of vitamin D and calcium on health outcomes has been proposed because of coherent observations in experimental and epidemiological studies. Calcium mediates vitamin D-induced apoptosis in breast cells[34] suggesting that parts of the apoptotic effects of vitamin D are made possible via calcium dependent processes. It has been shown that calcium deficiency promotes vitamin D deficiency and vitamin D requirements increased when dietary calcium reduces.[35] The randomised controlled trial conducted in United States also observed a reduced risk of total cancer among women on calcium supplementation but a more marked risk reduction among women on combined calcium + vitamin D supplementation. Likewise, signalling pathways for VDR and CaR converge on the same pathway giving rise to possibilities of interaction.[9]

4.4. *Methodological issues*

Our study has the following limitations. We did not have information on oral contraceptive use, family history of ovarian cancer and BMI which may influence our results; hence there may be residual confounding. It is however very likely that if the women were using oral contraceptives, they must have stopped for some months before the samples were was taken since they were pregnant women and the samples were taken between the 12th and 14th week of pregnancy. Also, our study subjects were limited to fertile women in their reproductive ages, hence we cannot generalise our results to infertile/and/or post-menopausal women.

The use of single-point biomarker measurement in determining risks may be questioned but studies have shown that there is a high individual correlation in 25-OHD concentrations over a 5 year period[36] and both 25-OHD and calcium exhibit low long-term intra-individual variations.[36,37] Likewise, we do not think pregnancy has affected the relationship between calcium, vitamin D and ovarian cancer because it has been shown that the serum calcium and 25-OHD concentrations are not appreciably different from the pre-pregnancy levels,[38] a result corroborated in our study whereby there was no correlation between gestational days and the two biomarkers.

In conclusion, calcium and vitamin D act independently to reduce ovarian cancer risk. While we observed a significant inverse association between calcium and ovarian cancer, the association between vitamin D and ovarian cancer was of borderline significance. The relationship between calcium and ovarian cancer is novel and intriguing, but needs to be validated in other studies as this could present new opportunities in ovarian cancer prevention.

Conflict of interest statement

None declared.

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