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Serum 25-hydroxyvitamin D and the risk of ovarian cancer ☆

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ABSTRACT

Introduction: Ecological and experimental studies suggest that vitamin D may be associated with a reduced risk of ovarian cancer. In this study, we sought to determine the risk of developing ovarian cancer according to serum 25-hydroxyvitamin D (25-OHD) concentrations assessed on average 5 years before the diagnosis.

Methods: We conducted a population-based longitudinal case–control study nested within the Finnish Maternity Cohort (FMC) which contains serum samples of virtually all pregnant women in Finland since 1983. Among them, 201 ovarian cancers diagnosed within 10 years of serum sampling were randomly selected as cases for this study. For each case, we selected two controls matched for age, parity and sampling season (±4 weeks) and one control matched for age and parity but for the opposite sampling season (6 months ± 4 weeks).

Results: The relative risks (estimated as odds ratio, OR) for ovarian cancer comparing the lowest quintile to the highest quintile of serum 25-OHD concentration were 1.8 (95% CI 0.9–3.5) among controls matched for the same season, and 1.1 (95% CI 0.6–2.2) among controls matched for the opposite season. The OR among women with insufficient (<75 nmol/L) serum 25-OHD concentration was 2.7 (95% CI 1.0–7.9, lower limit, 0.95) compared to that among those with sufficient (⩾75 nmol/L) serum 25-OHD concentration. No differences in the point estimates were observed between serous or mucinous histological subtypes of ovarian cancer.

Conclusion: Overall, we did not observe a significant association between serum 25-OHD concentrations and the risk of ovarian cancer. However, we found evidence suggestive of an increased risk among women with low to insufficient serum 25-OHD concentrations.

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1. Introduction

Ovarian cancer is the most lethal gynaecological malignancy and the fourth leading cause of cancer deaths among women worldwide.[1] About 191,000 new cases are diagnosed annually,[2] with the highest incidence rates found in Caucasians in northern and western Europe and in North America.[2] Despite its considerable burden to cancer mortality in women, very little is known about its aetiology.[1]

Ecological studies have suggested that there may be an association between solar ultraviolet B radiation and risk of ovarian cancer.[3–6] It has been postulated that the protective effects of sunlight on ovarian cancer are mediated via vitamin D, which is a steroid-like hormone mainly produced through the action of sunlight on the skin. Of the six ecological studies published regarding ovarian cancer mortality rates and solar radiation, four[3–6] reported higher mortality rates with lower regional sunlight whereas two observed no relationship.[7,8] Solar ultraviolet B (UVB) and high solar irradiance were also shown to be inversely associated with incidence rates of ovarian cancer in a worldwide study[9] while there was no association in another multinational study.[10] Likewise, epidemiological studies examining the association of dietary vitamin D and ovarian cancer have been equivocal.[11–14] However, one recent study that evaluated the association of plasma vitamin D and ovarian cancer risk reported a beneficial effect among certain subgroups.[15]

Vitamin D, by inducing cellular differentiation and apoptosis, inhibiting cellular proliferation, invasiveness and angiogenesis possesses anti-carcinogenic properties.[16] Vitamin D exerts its action through the vitamin D receptor (VDR) and VDRs are present in both normal ovarian tissues and ovarian cancer cells.[17–19]

In this nested case–control study, we examined the relationship between serum vitamin D concentration and risk of ovarian cancer exploiting the resources of the nationwide Finnish Maternity Cohort (FMC), established in 1983.[20]

2. Materials and methods

2.1. Finnish Maternity Cohort

We carried out a case–control study nested within the Finnish Maternity Cohort (FMC). The FMC was established by the National Institute for Health and Welfare, Finland in 1983.[20] 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 at −25 °C in polypropylene cryo vials in a well-protected biorepository at the National Institute for Health and Welfare in Oulu. More than 98% of pregnant women in Finland have donated blood samples to the cohort since 1983 and currently over 1.3 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.[21] Every resident of Finland has a unique personal identity code that is also used in official health registries such as the FMC and FCR. Our study cohort was record linked with the cancer registry data by using the personal identity code.

Two-hundred and one random ovarian cancer cases diagnosed within 10 years of serum sampling were selected for this study. If a case had donated more than one sample within this 10-year period, the sample donated closest to cancer diagnosis was selected. Time between serum donation and cancer diagnosis ranged between 2 years and 10 years with a median of 5 years. Among the 201 cancer cases, 195 had histological confirmation. Of the 195 with histological confirmation, 76 (39%) were serous, 70 (35.9%) mucinous, 15 (7.7%) sex cord stromal tumours, 11 (5.6%) endometrioid, 9 (4.6%) germ cell tumours, 5 (2.6%) clear cell tumours and 9 (4.6%) others. The tumours were left-sided in 74 (45.4%) cases, right-sided in 66 (40.5%) cases and bilateral in 23 (14.1%) cases.

The cases were matched with two sets of controls. The first set of controls consisted of women from the FMC who were alive and free of cancer at the time of diagnosis of the case and were matched for (i) age at sample withdrawal ±1 year, (ii) parity and (iii) date of index blood sampling ±4 weeks (same season as case). For each case, 2 controls were selected and 400 serum samples were available for the laboratory analysis. The second set of controls consisted of women from the cohort, who were alive and free of cancer at the time of diagnosis of the cases and whose serum samples were taken at the opposite seasons to the cases (approximately six months ± 4 weeks apart). These controls were matched for (i) age at sample withdrawal ±1 year and (ii) parity. One control per case was selected. In all, 201 cases, 398 same season controls and 199 opposite season controls were available.

All study samples were previously unthawed. The study was approved by the ethical committee of the National Institute for Health and Welfare, Finland.

2.3. Laboratory analysis

Quantification of 25-hydroxyvitamin D (25-OHD) was done at the Department of Medical Biosciences, University of Umeå, Umeå, Sweden using a 25-OHD radioimmunoassay (RIA) from IDS Ltd., Boldon, UK. 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% and 0.3% for cholecalciferol (D3) and ergocalciferol (D2), respectively. The within, between and total coefficients of variations (CVs) of the assay were 7.8%, 9.6% and 12.4% at level 28.4 nmol/L 25-OHD, and 4.1%, 7.4% and 8.5% at 107 nmol/L 25-OHD, respectively. Case and control samples belonging to each other study set were assayed together, ordered randomly and labelled to mask case–control status.

2.4. Statistical analysis

Two of the samples had 25-OHD concentrations of less than 2 nmol/L, which were considered to be outliers and set to missing. Descriptive data of cases and controls with regard

to age at sample withdrawal and cancer diagnosis, age at last full term pregnancy, number of pregnancies, gestational days and bench lag-time were recorded.

Quintile cut-off points were determined using 25-OHD concentrations of the controls. Quintiles of 25-OHD concentrations were used to estimate the relative risk of ovarian cancer and their 95% confidence intervals (CIs) by conditional logistic regression and are expressed as odds ratio (OR). The multivariate model was adjusted for age at last full term pregnancy and bench lag-time (days between sample withdrawal and freezing it for storage). Separate analyses were conducted for the main different histological subgroups of ovarian cancer available vis-a-vis, serous and mucinous tumours using cases and controls that were matched for the same season. Risk of ovarian cancer was also compared among women with serum 25-OHD levels above 75 nmol/L and those with serum 25-OHD levels below 75 nmol/L.[22,23] All statistical analyses were performed using SPSS 15 for windows (SPSS Inc., Chicago, IL). Two-sided $p < 0.05$ was considered statistically significant.

3. Results

The age range for cases and controls was from 17.5 to 44 years. The median ages at the time of serum sampling were 31.5 and 31.4 years for cases and controls, respectively. The median duration between serum sampling and cancer diagnosis was 5 years. The median number of pregnancies was 2 with a range of 1–8 for cases and 1–10 for controls. Median serum 25-OHD concentrations were 35.0, 35.8 and 34.3 nmol/L among cases, same season controls and opposite season controls, respectively (Table 1).

We first compared the relative risk of ovarian cancer by quintile groups of serum 25-OHD among cases and controls whose serum samples were taken within the same season and had been matched for age and parity. Low levels of 25-OHD appeared to be associated with increased risk of ovarian cancer. The strongest association did, however, not reach statistical significance (1st quintile versus 5th quintile: OR, 1.8, CI 0.9–3.6; $p_{trend} = 0.26$) age at last full term pregnancy and bench lag-time adjusted (Table 2). Women with insufficient serum vitamin D concentrations had a threefold increased risk of ovarian cancer compared to women with sufficient serum concentrations (OR 2.8, 95% CI 1.0–7.9, lower limit 0.95, p-value = 0.06) (Table 3). Different point estimates were obtained when control samples from opposite seasons were used. The point estimates were closer to unit risk (1st quintile versus 5th quintile: OR, 1.1, CI O.6–2.2; $p_{trend} = 0.49$) (Table 2).

No significant associations were observed between quintiles of 25-OHD concentrations and ovarian cancer risk when the analyses were carried out for the two main histological groups. Comparing the 1st to the 5th quintiles, the ORs were 1.3 (95% CI 0.7–3.2) for serous tumours, and 1.2 (95% CI 0.4–3.1) for mucinous tumours. Stratification for seasonality did not show any further differences (data not shown).

4. Discussion

In this longitudinal population-based nested case–control study, we observed no significant relationship between serum 25-hydroxyvitamin D concentrations and the risk of ovarian cancer. However, there appeared to be a tendency to increased risk among those who had low or insufficient serum 25-hydroxyvitamin D concentrations compared to those who had sufficient concentrations.

Perhaps, one of the reasons why an association between 25-OHD and ovarian cancer was not apparent using the quintile distribution of 25-OHD was the generally relatively low serum 25-OHD concentrations in the cohort. Recently, due to a greater understanding of the interplay between vitamin D and its regulatory hormones, there have been discussions on what constitutes vitamin D deficiency.[22–24] A 25-OHD concentration of less than 50 nmol/L is considered to represent vitamin D deficiency, and that between 50 and 72 nmol/L is considered to represent relative insufficiency while concentrations above 75 nmol/L are thought to indicate vitamin D sufficiency.[22] Using these cut-off values, we found evidence suggestive of an increased risk of ovarian cancer among women with insufficient serum vitamin D concentrations compared with women with sufficient vitamin D concentrations. This supports results from the experimental studies which suggest that high vitamin D concentrations may be needed to prevent against ovarian cancer.[25,26]

The results from our study are similar to those of another recent prospective study by Tworoger and colleagues.[15] The authors did not observe any association between plasma 25-

Table 1 – Baseline characteristics of the study population who donated serum samples to the Finnish Maternity Cohort between 1983 and 2006.

	Cases (n = 201) Median (range)	Control[a] (n = 398) Median (range)	Control[b] (n = 199) Median (range)
Age at serum sampling	31.5 (17.5–43.2)	31.5 (17.8–43.9)	31.4 (18.9–43.6)
Age at last full term pregnancy	32.7 (17.5–50.1)	33.6 (17.8–50.9)	33.2 (18.9–51.2)
Number of pregnancies	2 (1–8)	2 (1–10)	2 (1–10)
Lag-time to cancer diagnosis, years	5 (0.5–9.8)		
Duration of gestation, days	74.0 (40–89)	76.5 (33–91)	81.0 (35–87)
Bench lag-time, days	2.0 (0–12)	3.0 (0–9)	3.0 (0–9)
25-OHD concentration (10th and 90th percentiles), nmol/L	35.0 (20.3, 56.4)	35.8 (22.9, 61.3)	34.3 (21.5, 65.4)

a Same season controls. Serum samples were taken within 1 month to those of cases.

b Opposite season controls. Serum samples were taken during the opposite season to those of cases, i.e. approximately 6 months ± 4 weeks apart.

Table 2 – Relative risks (odds ratio, OR, with 95% confidence interval, CI) of ovarian cancer by quintile of serum vitamin D concentration in fertile aged Finnish women followed up for up to 10 years after serum withdrawal.

	Quintile 1	Quintile 2	Quintile 3	Quintile 4	Quintile 5	P_{trend}
Among cases and controls who donated serum in same season (±4 weeks)[a]						
Quintile values, nmol/L	<26.4	26.4–32.9	32.9–39.9	39.9–53.1	⩾53.1	0.26
n, cases, control	46/79	41/81	41/81	44/78	28/79	
OR, crude	1.6 (0.9–2.9)	1.4 (0.8–2.6)	1.4 (0.8–2.5)	1.6 (0.9–2.7)	1.0 (reference)	
OR, adjusted[b]	1.8 (0.9–3.5)	1.6 (0.8–3.1)	1.5 (0.8–2.8)	1.6 (0.9–2.9)	1.0 (reference)	
Among cases and controls who donated serum in opposite seasons (6 months ± 4 weeks)[a]						
Quintile values, nmol/L	<25.3	25.3–32.2	32.2–38.8	38.8–51.9	⩾51.9	0.49
n, cases, control	42/39	36/41	41/39	41/39	41/40	
OR, crude	1.1 (0.6–2.0)	1.3 (0.7–2.3)	1.1 (0.6–2.0)	1.1 (0.6–2.0)	1.0 (reference)	
OR, adjusted[b]	1.1 (0.6–2.2)	1.3 (0.6–2.5)	1.2 (0.6–2.3)	1.2 (0.6–2.3)	1.0 (reference)	

a Cases and controls were also matched for parity and age.
b Adjusted for age at last full term pregnancy and bench lag-time.

Table 3 – Relative risks (odds ratio, OR, with 95% confidence interval, CI) of ovarian cancer in women with sufficient serum vitamin D concentrations compared with those of fertile aged Finnish women with insufficient serum vitamin D concentrations followed up for up to 10 after serum withdrawal.[a]

	Cases	Controls	OR	OR, adjusted (95% CI)	P-value
Sufficient ⩾ 75 nmol/L (reference)[b]	4	21	1.0		0.06
Insufficient (<75 nmol/L)	196	377	2.8	2.7 (1.0[c]–7.9)	

a Analysis carried out among cases and controls matched for age, parity and season of serum donation (±4 weeks).
b Refs. 22,23.
c Lower limit, 0.95.

hydroxyvitamin D and ovarian cancer risks in their overall analysis but women with adequate versus inadequate 25-hydroxyvitamin D levels had a decreased risk of borderline significance (RR, 0.7; 95% CI, 0.4–1.1). In addition, Tworoger and colleagues reported a stronger effect among overweight women. Unfortunately, data on body weight were not available for the women in our sub-sample of the FMC to explore this hypothesis.

The ovary together with other tissues such as the kidney, breast, colon and prostate express the enzyme, 1 alpha-hydroxylase, (1aOHase).[25] This enzyme converts 25-OHD to 1, 25-$(OH)_2$D, thus, the biologically active form of vitamin D is available locally in the ovaries and other tissues that express the enzyme. Since the ovaries also express VDR[18,19] and the effects of vitamin D are exerted through the VDR, it is biologically plausible that vitamin D may have effects on the ovaries.

Ovarian cancer is a heterogeneous disease. The histological subtypes have different risk factors (both genetic and environmental) and dissimilar epidemiological and clinical characteristics.[27] In our study, we did not observe any differences in risk related to vitamin D concentration by histology. Apart from serous and mucinous cancers, the other histological subgroups, however, had very few cases.

The following limitations of our study need to be taken into account: (i) oral contraceptive use increases serum 25-OHD concentrations[28,29]; and use of oral contraceptives also protects against ovarian cancer.[30] Hence, use of oral contraceptives is a confounder in the association between 25-OHD and ovarian cancer. While this was controlled for by sample withdrawal during 12th to 14th week of pregnancy (when use of oral contraceptive must have been stopped for sometime), we did not have information on the use of oral contraceptives among our study subjects. (ii) Our study subjects were limited to fertile women in their reproductive ages who were matched for parity. Therefore we do not know if similar results will be obtained in infertile and/or post-menopausal women since they were not represented in our study.

Serum 25-OHD concentration is a reflection of endogenous synthesis taking place in the skin under the influence of UVB irradiation and exogenous production from dietary intake.[22,31] Vitamin D first undergoes hydroxylation in the liver to 25-hydroxyvitamin D (25-OHD) before undergoing further hydroxylation to 1, 25-dihydroxyvitamin D (1, 25-$(OH)_2$D) in the kidneys.[22] While 1, 25-$(OH)_2$D is the most biologically active form of vitamin D, 25-OHD is considered as the best gauge of individual vitamin D status,[22,31,32] making measurement of serum/plasma 25-OHD concentrations the ideal way to determine vitamin D status.

Strengths of our study include (i) its population-based nature; the FMC covers 98% of pregnant Finnish women.[20] (ii) Its prospective nature; the blood samples were collected before cancer diagnosis. Furthermore, the study samples were also collected before the start of vitamin D supplementation among pregnant women in Finland. In addition, we were able to explore the possible effects of matching for season of blood collection on the results. The reason why we selected the opposite season control group was to determine how much variation in risk estimates can occur when such controls are used as compared to when same season controls are used.

We believe that this may have important repercussions on studies examining vitamin D/disease associations especially in serial sample studies. In this study, deliberate opposite season controls showed lower, close to unit risk estimates when compared to season-matched controls. This could mean that if an effect exists, not taking into account the input of seasonality probably confounds the estimation. In epidemiological studies ascertaining vitamin D disease associations, adequate matching of cases and controls for time of sample collection and/or statistical control is essential.

In conclusion, although we did not observe statistically significant association between serum 25-OHD concentrations and risk of ovarian cancer, low serum 25-OHD concentrations may be associated with an increased risk of developing the disease. As few modifiable factors are known to reduce ovarian cancer risk, further prospective data on vitamin D and ovarian cancer are of interest.

Conflict of interest statement

None declared.

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