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Prospective studies of total and ionized serum calcium in relation to incident and fatal ovarian cancer

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HIGHLIGHTS

- Two independent nationally representative population-based cohorts are examined.
- Higher ionized and total serum calcium are associated with ovarian cancer mortality.
- Confirmation that higher total serum calcium is associated with incident ovarian cancer in a second cohort

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ABSTRACT

Objective. Biological markers that could aid in the detection of ovarian cancer are urgently needed. Many ovarian cancers express parathyroid hormone-related protein, which acts to raise calcium levels in serum. Thus, we hypothesized that high serum calcium levels might predict ovarian cancer.

Methods. We examined the associations between total and ionized serum calcium and ovarian cancer mortality in the Third National Health and Nutrition Survey (NHANES III) using Cox proportional hazard models. We then examined the associations of serum calcium with incident ovarian cancer in a second prospective cohort, the NHANES Epidemiological Follow-up Study (NHEFS).

Results. There were eleven deaths from ovarian cancer over 95,556 person-years of follow-up in NHANES III. After multivariable adjustment, the risk for fatal ovarian cancer was 52% higher for each 0.1 mmol/L increase in total serum calcium (RH = 1.52, 95% CI 1.06–2.19) and 144% higher for each 0.1 mmol/L increase in ionized serum calcium (RH = 2.44, 95% CI = 1.45–4.09). Associations persisted after adjusting for nulliparity and the use of oral contraceptives. Eight incident ovarian cancers occurred over 31,089 person-years of follow-up in the NHEFS. After adjusting for covariates, there was a 63% higher risk for ovarian cancer with each 0.1 mmol/L increase in total serum calcium (95% CI 1.14–2.34). Similar results were observed for albumin-adjusted serum calcium.

Conclusions. Higher serum calcium may be a biomarker of ovarian cancer. This is the first report of prospective positive associations between indices of calcium in serum and ovarian cancer. Our findings require confirmation in other cohorts.

Introduction

Ovarian cancer is the most fatal of the gynecologic cancers. The high fatality rate results from the late stage of presentation, at which time ovarian cancers have metastasized and their curability is low. In theory, early diagnosis of ovarian cancer might be accomplished through the use of biomarkers in blood or urine. However, the most widely studied serum marker for ovarian cancer, CA-125, is elevated in only 50% of women with curable (Stage 1) disease [1]. Consequently, there is great interest in the discovery of additional biomarkers that could help to detect ovarian cancers at a curable stage.

One approach to cancer biomarker discovery is to identify a factor(s) that is differentially expressed in individuals with and without cancer and to examine that factor's ability to detect cancer in an independent sample of individuals with and without cancer [2,3]. Many ovarian cancers express increased levels of parathyroid hormone-related protein (PTHrP), an oncofetal protein that is the principal agent of hypercalcemia of malignancy [4]. PTHrP acts to increase the release of

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calcium from bone and to retard the excretion of calcium in the kidney, causing calcium levels in serum to rise [5]. Although only a small minority of ovarian cancers are characterized by hypercalcemia (i.e., serum calcium levels > upper limit of the normal reference range), the evolution of hypercalcemia in ovarian cancer may be gradual. That is, tumors likely evolve from normocalcemia to high normocalcemia before causing hypercalcemia. Thus, we hypothesized that high serum calcium levels might detect ovarian cancer at a preclinical phase.

We tested this hypothesis using data on serum calcium in two nationally representative prospective cohorts, the Third National Health and Nutrition Examination Surveys (NHANES III) and the NHANES Epidemiologic Follow-up Study (NHEFS).

Methods

Baseline data and serum samples were collected as part of NHANES III between 1988 and 1994 [6]. Total and ionized serum calcium levels were measured using ion-specific electrodes and were pH-adjusted. Because the protein binding of calcium is affected by pH, ionized calcium in blood is commonly corrected to standard pH [7]. Approximately half of total serum calcium is in the "free" or ionized state; approximately 40% is bound to serum proteins, principally albumin, and the remainder is bound to anions. Ionized serum calcium is the biologically active fraction of total serum calcium. Because the measurement of ionized calcium is technically more challenging and more expensive than the measurement of total serum calcium, ionized calcium levels often are estimated by calculating serum calcium levels adjusted for serum albumin. We computed levels of albumin-adjusted serum calcium for women with a serum albumin below 4.0 g/dL using a standard formula (0.8 times the difference between 4.0 g/dL and the observed albumin, plus the observed total serum calcium in mg/dL) [8].

The outcome in NHANES III was death attributed to ovarian cancer on the death certificate with mortality linkage through December 31, 2006. Follow-up time was computed as the number of months between the baseline exam and death from ovarian cancer (events) or any other cause (censored), or December 31, 2006 if participants were alive. We excluded women who reported that they had no ovaries at baseline but included women with a prior personal history of non-ovarian cancers because they remain at risk for ovarian cancer. No follow-up for incident cases was performed for NHANES III and information about sub-types of ovarian cancer was not available.

We conducted a second, confirmatory, population-based prospective study using the first NHANES Epidemiologic Follow-up Study (NHEFS) with incident ovarian cancer as the outcome. We included women ages 25 to 75 years at the baseline examination in 1971 to 1975. Follow-up questionnaires for incident medical conditions were administered in 1982, 1986, 1987, and 1992. Women who reported having no ovaries at baseline were excluded but women with a prior personal history of non-ovarian cancers were included, as in NHANES III. We computed follow-up time as the interval between baseline examination and date of diagnosis with ovarian cancer (events) or a report of removal of both ovaries (censored), or the end of follow-up in December 1992 [9–12]. Data on ionized calcium were not available in NHEFS.

We used Cox proportional hazard regression models accounting for survey weights and the complex sampling design to estimate relative hazards and 95% confidence intervals (CI) for ovarian cancer death by incremental (0.1 mmol/L) differences in total serum calcium, albumin-adjusted serum calcium, and ionized serum calcium. We examined potential confounding by age, height, body mass index (BMI), race/ethnicity (Black versus all other), cigarette smoking status (ever vs. never), nulliparity (no live births versus any), and use of oral contraceptives (ever vs. never). Statistical analyses were performed using R v 2.15.0 with the "survival" package [13].

Results

Table 1 shows selected characteristics of women in NHANES/NHEFS and NHANES III by categories of total serum calcium at baseline.

Eleven ovarian cancer deaths were observed over 95,556 person-years of follow-up through December 31, 2006, representing 137,404 ovarian cancer deaths in the United States. The range in total serum calcium in cases was 2.14 to 2.44 mmol/L and for ionized serum calcium was 1.17 to 1.31 mmol/L. The normal reference range for total serum calcium is approximately 2.17 to 2.52 mmol/L [8.7 to 10.1 mg/dL] and 1.12 to 1.32 mmol/L [4.5 to 5.3 mg/dL] for ionized serum calcium [14]. The range of times from calcium measurement to death was 28 to 208 months. In multivariable Cox models, the relative hazard for fatal ovarian cancer was 1.52 per 0.1 mmol/L increase in total serum calcium (95% CI 1.06–2.19) and 2.44 per 0.1 mmol/L increase in ionized serum calcium (95% CI 1.45–4.09). Adjustment for race, cigarette smoking, height and BMI did not materially alter the estimates vs. age-adjusted estimates. Further adjustment for nulliparity and the never use of oral contraceptives yielded relative hazards (RHs) of 1.46 (1.02–2.09) and 2.11 (1.16–3.83) for total and ionized serum calcium, respectively. Due to the small number of events, we could not explore the interactions between serum calcium concentration and covariates (Table 2).

We sought to confirm these findings using a second prospective population-based cohort, the NHEFS. There were 8 incident ovarian cancer cases in the NHEFS over 31,089 person-years of follow-up. The range of total serum calcium was 1.98 to 2.93 mmol/L. The range of times from calcium measurement to diagnosis with ovarian cancer was 12 to 240 months. The multivariable adjusted relative hazard for ovarian cancer for each 0.1 mmol/L increase in total serum calcium was 1.63 (95% CI 1.14–2.34). Adjusting for BMI, height, and cigarette smoking status did not materially change the association compared to adjusting for age alone. Further adjustment for nulliparity and ever use of oral contraceptives moderately

Table 1
Selected characteristics of women in the First National Health and Nutrition Examination Survey (NHANES), NHANES Epidemiology Follow-up Study (NHEFS) and Third National Health and Nutrition Examination Survey (NHANES III) by tertile of serum total calcium concentration at baseline.

	Baseline total serum calcium tertile in NHANES/NHEFS					
Total calcium range (mmol/L)	1.98–2.38		2.38–2.45		2.45–2.93	
Number of participants	725		507		516	
Weighted population	19,842,226		13,751,362		13,913,528	
Ovarian cancer cases through 1992	1		1		6	
Person-months of follow-up	158,280		106,800		107,988	
Mean total calcium (mmol/L)	9.27	(0.08)	9.69	(0.02)	10.13	(0.08)
Mean age (years)	45.80	(16.81)	46.50	(17.77)	47.75	(19.01)
Mean body mass index (kg/m^2)	25.17	(6.40)	25.45	(6.10)	25.42	(6.19)
Mean albumin (g/dL)	4.20	(0.39)	4.35	(0.32)	4.46	(0.31)
	Baseline total serum calcium tertile in NHANES III					
Total calcium range (mmol/L)	1.57–2.26		2.26–2.34		2.34–3.29	
Number of participants	2644		2219		2127	
Weighted population	28,375,219		24,631,015		21,193,034	
Ovarian cancer deaths through 2006	3		4		4	
Person-months of follow-up	442,665		262,210		341,803	
Mean total calcium (mmol/L)	2.20	(0.08)	2.31	(0.02)	2.43	(0.08)
Mean ionized calcium (mmol/L)	1.21	(0.04)	1.24	(0.03)	1.27	(0.05)
Mean age (years)	42.53	(16.81)	42.76	(17.77)	44.74	(19.01)
Mean body mass index (kg/m^2)	26.25	(6.40)	26.20	(6.10)	26.40	(6.19)
Mean albumin (g/dL)	3.97	(0.39)	4.11	(0.32)	4.22	(0.31)

Means and standard deviations account for the complex sampling design and survey weights in NHANES/NHEFS and NHANES III. Where means are given, standard deviations appear in parentheses.