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Circulating Prolactin Levels and Risk of Epithelial Ovarian Cancer

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Abstract

Purpose—Indirect evidence from experimental and epidemiological studies suggests that prolactin may be involved in ovarian cancer development. However, the relationship between circulating prolactin levels and risk of ovarian cancer is unknown.

Methods—We conducted a nested case-control study of 230 cases and 432 individually-matched controls within three prospective cohorts to evaluate whether pre-diagnostic circulating prolactin

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is associated with subsequent risk of ovarian cancer. We also assessed whether lifestyle and reproductive factors are associated with circulating prolactin among controls.

Results—Prolactin levels were significantly lower among post- vs. pre-menopausal women, parous vs. nulliparous women, and past vs. never users of oral contraceptives in our cross-sectional analysis of controls. In our nested case-control study, we observed a non-significant positive association between circulating prolactin and ovarian cancer risk (OR_{Q4vsQ1}: 1.56, 95% CI: 0.94, 2.63, p-trend: 0.15). Our findings were similar in multivariate-adjusted models and in the subgroup of women who donated blood ≥5 years prior to diagnosis. We observed a significant positive association between prolactin and risk for the subgroup of women with BMI ≥25 kg/m^2 (OR_{Q4vsQ1}: 3.10, 95% CI: 1.39, 6.90), but not for women with BMI <25 kg/m^2 (OR_{Q4vsQ1}: 0.81, 95% CI: 0.40, 1.64).

Conclusions—Our findings suggest that prolactin may be associated with increased risk of ovarian cancer, particularly in overweight/obese women. Factors associated with reduced risk of ovarian cancer, such as parity and use of oral contraceptives, were associated with lower prolactin levels, which suggests that modulation of prolactin may be a mechanism underlying their association with risk.

Keywords

Prolactin; Ovarian Cancer; Serum; Plasma

Background

The polypeptide hormone prolactin has numerous functions in addition to its important role in lactation, including a role in reproduction (*i.e.,* maintaining normal ovarian function and modulating the effects of gonadotropins) and modulating immune function [1, 2]. Though prolactin is primarily produced in the pituitary gland it is also produced in other tissues, including the ovaries [1]. The prolactin receptor is expressed in normal ovarian and fallopian tube tissues [1, 3, 4], the primary sites of origin for ovarian tumors. There are several ways that prolactin could influence ovarian cancer development. Animal and *in vitro* studies have shown that prolactin promotes growth of ovarian surface epithelial cells and inhibits apoptosis and increases survival of ovarian cancer cells [5-8]. Furthermore, prolactin levels increase in response to psychosocial and physical stress [9], which was associated with greater tumor burden and tumor invasiveness in a mouse model of ovarian cancer [10, 11]. In cross-sectional studies, known risk factors for ovarian cancer (e.g. nulliparity and endometriosis) were associated with higher prolactin levels [12, 13], which suggests that prolactin may be part of the underlying mechanism through which these factors influence risk. Prolactin receptor expression and circulating prolactin levels have been shown to be higher among women with ovarian cancer vs. benign-condition or healthy controls [6, 14, 15]. However, a major limitation of these retrospective studies is that prolactin levels may have been affected by the presence of the tumor and/or the stress associated with cancer diagnosis or treatment. Thus, the purpose of this study was to assess prospectively the relationship between pre-diagnostic circulating levels of prolactin and subsequent risk of invasive ovarian cancer. We also performed a cross-sectional analysis in controls to examine factors associated with prolactin levels.

Methods

We conducted a nested case-control study within three prospective cohorts, the NYU Women's Health Study (NYUWHS), the Northern Sweden Health and Disease Study (NSHDS), and the ORDET cohort in Italy. These parent cohorts and nested case-control study of epithelial ovarian cancer have been described previously [16]. In total, 230 ovarian

cancer cases and 432 controls (~2 per case matched on age, menopausal status, and date of blood sampling) were included. Prolactin was measured using Luminex Xmap multiplex bead-based technology using a kit from Linco/Millipore according to the manufacturer's instructions. All prolactin values were above the limit of detection of the assay. Blinded replicates from a serum pool were used for quality control and were interspersed at random on each plate. The intra- and inter-batch coefficients of variation for prolactin were ≤ 1%.

Prolactin values were log transformed to reduce departure from the normal distribution. Seven outliers (3 cases and 4 controls) were identified using the generalized extreme Studentized deviate many-outlier procedure described by Rosner [17]. Removal of these outliers did not change the results appreciably. To assess the relationship between lifestyle/reproductive factors and prolactin levels, we performed a cross-sectional analysis in the controls, using generalized estimating equations to calculate geometric means adjusted for cohort and age (continuous), taking into account the correlation between controls from the same matched set. We also performed cross-sectional analyses mutually adjusted for all factors significantly associated with prolactin levels in our study (age, parity, oral contraceptive use, and menopausal status). To examine the association between prolactin levels and ovarian cancer risk, conditional logistic regression, which takes into account the risk set sampling and matching factors (age, menopausal status, and sample storage time), was used to estimate odds ratios across quartiles of prolactin. Quartile cut points were based on the cohort-specific distribution of values in the cases and controls combined. Multivariate conditional logistic regression models were adjusted for parity (ever/never had a full term pregnancy) and use of oral contraceptives (OCs, past/never). There were no current users of OCs because the study eligibility criteria required that women were not using any exogenous hormones (OCs or hormone replacement therapy) at the time of blood donation. Missing data for parity (9.2% of women) and OC use 14.4% of women) were imputed for each cohort separately using a fully conditional specification (FCS) multiple imputation method [18], as appropriate for the imputation of missing categorical variables, adjusted for case-control status. Other known and suspected risk factors for ovarian cancer, including family history of breast or ovarian cancer and body mass index (BMI), were not included in the final model because they were not associated with prolactin levels and their inclusion did not affect the odds ratio estimates appreciably (<10% change in the odds ratios) (data not shown). To test for cohort heterogeneity, we compared the model with the cross product term (prolactin × cohort) to the model without this term using the likelihood ratio test, and we also used the Q statistic. To increase the effective sample size for the analysis stratified by BMI, OC use, and parity, we broke the case-control matching and controlled for the matching factors in unconditional logistic regression models, after verifying that these two methods produced very similar odds ratios.

Results

Characteristics of cases and controls are shown in Table 1. The median age at blood sampling (at the time of cohort enrollment) was 55 years. The time between blood donation and diagnosis was ≥5 years for 65% of cases. Although differences were not statistically significant, cases were more likely to have a family history of breast or ovarian cancer (23% vs. 15%, p=0.07), more likely to be nulliparous (23% vs. 18%, p=0.17), and less likely to have used OCs (64% vs. 70%, p=0.26), as expected.

Table 2 shows the geometric mean prolactin by categories of lifestyle and reproductive factors. In cohort-adjusted models, prolactin levels decreased significantly with age (p-trend ≤ 0.001). Adjusting for age and cohort, prolactin was lower among post-vs. pre-menopausal women (13.3 vs. 17.8, p ≤ 0.001), ever-parous vs. nulliparous women (14.3 vs. 17.0, p = 0.01), and past vs. never users of OCs (13.6 vs. 15.5, p=0.03). Simultaneous adjustment for

all of the factors that were significantly associated with prolactin levels (*i.e.,* age, menopausal status at enrollment, parity, and use of OCs), did not change the geometric means appreciably, though the trend by age was no longer significant due to adjustment for menopausal status (p-trend = 0.08, p-value for difference between means = 0.03). The association between prolactin and all reproductive and lifestyle factors was similar for pre- and post-menopausal women in stratified analyses (data not shown).

Odds ratios and 95% confidence intervals showing the relation between prolactin levels and ovarian cancer risk are presented in Table 3. We observed a modest increase in risk for the highest vs. the lowest quartile of prolactin in unadjusted analyses (OR for the highest vs. lowest quartile: 1.56, 95% confidence interval: 0.94, 2.63), though the association and test for trend across quartiles (p = 0.15) was not statistically significant. Results were similar after adjustment for parity (ever/never) and OC use (past/never). Among the subgroups of women with more detailed information about number of full term pregnancies, age at first full term pregnancy, and duration of OC use, adjustment for these variables did not result in an appreciable change in the ORs compared with models adjusted for dichotomized parity (ever/never) and OC use (past/never). The test for heterogeneity by cohort was not significant (p=0.33 for both the likelihood ratio and Q tests). We observed a significant trend of increasing risk across quartiles of prolactin in the subgroup of women with BMI ≥25 kg/m^2 (OR_{Q4vQ1}: 3.10, 95% CI: 1.39, 6.90, p-trend=0.01), but no association for women with BMI <25 kg/m^2 (OR_{Q4vQ1}: 0.81, 95%CI: 0.40, 1.64, p-trend=0.46, p-interaction = 0.06; Table 3). Odds ratios for prolactin were not appreciably different in subgroups defined by menopausal status at enrollment (pre/post), parity (nulliparous/parous), or OC use (past/never). There was no evidence of interaction by time to diagnosis (<5/≥5 years after blood donation). The association between prolactin and risk in analyses restricted to women with the serous tumor subtype was in the same direction as the overall results.

Discussion

In this prospective case-control study nested within three cohorts, we observed a non-significant trend of increasing risk of ovarian cancer across quartiles of circulating prolactin. Odds ratios were similar for women who were diagnosed five or more years after blood donation, suggesting that the association we observed was not due to the effect of disease on prolactin levels. Results for the subgroup of women with the serous histological tumor subtype were also in the same direction as the overall results. Prolactin was significantly associated with ovarian cancer risk for women with BMI ≥ 25 kg/m^2, but not for women with lower BMI (p-interaction=0.06).

In our cross-sectional analysis in controls, we confirmed that prolactin is inversely associated with age. Consistent with the literature, we found that prolactin levels were lower in post-menopausal vs. premenopausal women [13, 19-21] and among parous vs. nulliparous women [13, 20, 22-31], and that prolactin levels did not vary by age at first birth [13, 20, 24, 27, 30, 31] or age at menarche [13, 27, 29].

To our knowledge, only two previous studies have assessed the relationship between history of OC use and prolactin levels: premenopausal [31], but not postmenopausal [27], ever users of OCs were shown to have lower prolactin levels than never users. In our study, past use of OCs was associated with lower prolactin levels in both pre- and post-menopausal women. Many changes in OC formulations have occurred over time and there are numerous formulations on the market with varied doses and types of estrogens and progestins, thus it is difficult to compare results across studies. If OC use is associated with reduced prolactin levels, as our data and that of Wang, et al [31] suggest, this may partially explain the

consistent protective effect of OC use on ovarian cancer risk, though further studies are needed to assess this mechanism.

Though women with a family history of breast and/or ovarian cancer had higher prolactin levels in some studies [13, 32-35], most studies [20, 22, 27, 31, 36-39], including ours, did not observe any difference in prolactin among women with vs. without a family history.

Prolactin and the prolactin receptor are expressed in ovarian and fallopian tube tissues and are involved in physiological ovarian processes, such as follicle development and corpus luteum function, during the menstrual cycle as well as pregnancy [1, 3, 4]. Experimental evidence suggests that increased prolactin signaling, through upregulation of the prolactin receptor, may play a role in promoting cancer development by increasing cell proliferation, reducing apoptosis, and modulating immune function [1, 5, 6]. Other carcinogenic mechanisms of prolactin have been suggested, including increased angiogenesis and cell motility as well as cross-talk with inflammatory mediators [reviewed in [6]]. Several retrospective studies have reported higher levels of circulating prolactin among women with ovarian cancer vs. benign-condition or healthy controls [6, 14, 15]. To our knowledge, no other prospective studies have assessed the relationship between pre-diagnostic circulating prolactin and subsequent risk of ovarian cancer. Our results suggest that prolactin may be associated with increased risk of ovarian cancer, although the odds ratios and tests for trend across quartiles were not significant.

We observed that prolactin was significantly associated with risk for women with BMI ≥ 25 kg/m^2, but not for women with BMI <25 kg/m^2, in models adjusted for age, menopausal status, parity, OC use, and BMI. Obesity is considered to be a low grade chronic inflammatory state [40], thus high prolactin levels among women with higher BMI may act in combination with increased levels of inflammation mediators to modulate immune function [1, 2] and contribute to ovarian cancer development. Despite the biological plausibility of our finding, our BMI subgroup analysis was based on a fairly small number of women, and the association may be due to chance. The distribution of ovarian cancer histological subtypes in each BMI subgroup was similar.

A limitation of our study is that circulating levels of prolactin may not reflect local levels in ovarian cancer tissues of origin, *i.e.,* the ovaries and fallopian tubes. Local prolactin levels may be a more relevant exposure because prolactin can act as both an endocrine and paracrine signaling molecule [1]. Measuring prolactin levels in the ovaries or fallopian tubes, though, is not an option in prospective studies of healthy subjects such as ours. However, data on the correlation between local and circulating prolactin levels from other studies, for instance in women undergoing tubal ligation, would help interpret the results of studies on circulating levels. Furthermore, several prolactin receptor isoforms have been identified in the ovaries and the varied expression and dimerization of these receptors may influence the effects of the prolactin ligand on ovarian cancer risk [41]. Studies have shown that there are also several variant forms of prolactin [42, 43]. Our immunoassay was not able to distinguish between the different isoforms or structural variants of prolactin which may have different bioavailabilities and biological actions. However, the prolactin variant(s) most likely to be relevant to ovarian cancer development have not yet been identified.

We only had one measurement of prolactin for each woman. However, we observed high temporal reliability (intra-class correlation coefficient of ~0.6-0.7 in serum and plasma) in paired serum samples from the NYUWHS cohort (n=65 pairs) and plasma samples from the NSHDS cohort (n=18 pairs), collected from the same women over a 2-3 year period ([44] and unpublished data). These results indicate that a single prolactin measurement is representative of a woman's average level over a few years' time. The fairly high ICCs,

despite the fact that we did not control for the effects of time of day of sample collection, fasting status, and medication use, which have been shown to affect levels of prolactin [31, 45-48], suggest that the intra-subject variability due to these factors is not large, relative to the between subject variability. Although one study reported low reproducibility for prolactin among premenopausal women in the luteal phase of the menstrual cycle (ICC: 0.41) [48] and another reported low reproducibility among pre-(ICC: 0.40) and post-menopausal (ICC: 0.18) women [49], most other studies have reported high reproducibility estimates (ICC range: 0.53-0.76) for pre- and post-menopausal women similar to the present study [48, 50, 51], including an earlier study within the NYUWHS cohort that used a different assay method [50].

In summary, we observed a modest positive association between prolactin and risk of ovarian cancer, though it was not statistically significant except for the subgroup of women with BMI ≥ 25 kg/m^2. Consistent with the literature, we observed an inverse association between prolactin levels and both parity and OC use, suggesting that reduced prolactin secretion may be one mechanism underlying the inverse association between these factors and ovarian cancer risk.

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Table 1

Characteristics of ovarian cancer cases and matched controls, NYUWHS, NSHDS, and ORDET

Characteristics	Cases (N=230)	Controls (N=432)	p-value
Age at blood sampling, y, n (%)			
≤45 years	48 (20.9)	90 (20.9)	
46-55 years	73 (31.7)	134 (31.1)	
>55 years	109 (47.4)	207 (48.0)	Matched
Time to diagnosis, y, n (%)			
<5	80 (34.8)		
≥5	150 (65.2)		
Age at menarche, y, n (%)			
<13	72 (35.0)	145 (36.0)	
≥13	134 (65.0)	258 (64.0)	0.95
Unknown, n (% of total missing)	24 (10.4)	29 (6.7)	
Body Mass Index, kg/m^2, n (%)			
<25	115 (53.5)	204 (49.8)	
≥25	100 (46.5)	206 (50.2)	0.53
Unknown, n (% of total missing)	15 (6.5)	22 (5.1)	
Menopausal status at baseline, n (%)			
Premenopausal	86 (37.6)	161 (37.4)	
Postmenopausal	143 (62.4)	270 (62.6)	Matched
Family history of breast or ovarian cancer, n (%)			
No	125 (77.6)	270 (84.9)	
Yes	36 (23.4)	48 (15.1)	0.07
Unknown [a], n (% of total missing)	69 (30.0)	114 (26.4)	
Parity, n (%)			
Nulliparous	45 (22.5)	70 (17.5)	
Parous	155 (77.5)	331 (82.5)	0.17
Unknown, n (% of total missing)	30 (13.0)	31 (7.2)	
Age at first full term pregnancy [b], y, n (%)			
< 25 years	45 (45.5)	95 (45.2)	
≥ 25 years	54 (54.5)	115 (54.8)	0.78
Unknown, n (% of total missing)	56 (36.1)	121 (36.6)	
Use of oral contraceptives, n (%)			
Never	139 (69.8)	237 (64.4)	
Past	60 (30.2)	131 (35.6)	0.26
Unknown, n (% of total missing)	31 (13.5)	64 (14.8)	
Histology, n (%)			
Serous	120 (52.2)		
Endometrioid	30 (13.0)		
Clear cell	16 (7.0)		
Mucinous	22 (10.0)		

Characteristics	Cases (N=230)	Controls (N=432)	p-value
Undifferentiated	8 (3.5)		
Not Otherwise Specified	21 (9.1)		
Unknown	13 (5.7)		
Prolactin, ng/mL, median (25^{th}, 75^{th})	15.6 (8.5, 29.7)	14.8 (8.1, 29.8)	0.39

[a] Variable is not available from the ORDET cohort (41 cases and 82 controls)

[b] Among ever parous women

Table 2

Factors associated with prolactin levels among healthy controls

Factor	N	Geometric Mean, 95% CI, ng/mL [a]	p-value	N	Geometric Mean, 95% CI, ng/mL [b]	p-value
Age						
≤45 years	87	19.1 (17.2, 21.3)		74	16.3 (13.6, 19.5)	
46-55 years	133	16.9 (15.3, 18.7)		106	16.3 (14.7, 18.2)	
>55 years	207	12.3 (11.5, 13.2)	<0.001 [d]	178	13.4 (12.1, 14.8)	0.08 [d]
Age at menarche						
<13 years	143	15.3 (14.0, 16.7)		129	15.0 (13.7, 16.5)	
≥13 years	256	14.5 (13.6, 15.5)	0.32	225	14.7 (13.7, 15.7)	0.65
BMI						
<25 kg/m^2	202	15.2 (14.1, 16.4)		170	15.3 (14.2, 16.5)	
≥25 kg/m^2	204	14.6 (13.5, 15.7)	0.41	172	14.3 (13.2, 15.6)	0.23
Menopausal status						
Premenopausal	156	17.8 (15.8, 20.1)		130	17.6 (15.4, 20.2)	
Postmenopausal	270	13.3 (12.3, 14.3)	<0.001	228	13.4 (12.3, 14.5)	0.005
Family history of breast or ovarian cancer						
No	267	14.8 (13.9, 15.8)		233	14.8 (13.9, 15.8)	
Yes	48	14.1 (12.2, 16.2)	0.52	42	14.4 (12.4, 16.8)	0.72
Parity						
Nulliparous	68	17.0 (15.2, 19.1)		54	16.7 (14.8, 18.9)	
Parous	329	14.3 (13.5, 15.3)	0.01	304	14.4 (13.5, 15.4)	0.03
Age at first FTP [c]						
<25 years	95	13.6 (12.4, 15.0)		81	13.9 (12.6, 15.3)	
≥25 years	114	14.6 (13.3, 16.0)	0.31	103	15.0 (13.6, 16.4)	0.30
OC use						
Never	235	15.5 (14.5, 16.6)		231	15.5 (14.5, 16.5)	
Past	129	13.6 (12.2, 15.2)	0.04	127	13.6 (12.2, 15.2)	0.04

[a] Models adjusted for cohort (NYUWHS, ORDET, NSHDS) and age (continuous); note: age model is not adjusted for age (continuous)

[b] Models adjusted for cohort (NYUWHS, ORDET, NSHDS), age (continuous), menopausal status, parity, and OC use; note: models were not adjusted for covariate when it was included as the predictor (e.g. age model is not adjusted for age).

[c] FTP = full term pregnancy, among parous women

[d] p-value for trend

Table 3

Odds ratios and 95% confidence intervals for prolactin and ovarian cancer risk

	Cases (n)	Controls (n)	Quartiles of Prolactin				p-trend	p-interaction [e]
			Quartile 1	Quartile 2	Quartile 3	Quartile 4		
All women, unadjusted model [a]	230	432	1.0 (ref)	1.33 (0.83, 2.12)	1.14 (0.69, 1.86)	1.56 (0.94, 2.63)	0.15	-
All women, adjusted model [b]	230	432	1.0 (ref)	1.32 (0.82, 2.12)	1.07 (0.65, 1.76)	1.48 (0.88, 2.49)	0.25	-
Subgroups								
BMI [c]								
<25 kg/m^2	115	203	1.0 (ref)	0.81 (0.40, 1.64)	0.59 (0.29, 1.19)	0.81 (0.40, 1.64)	0.46	0.06
≥25 kg/m^2	99	206	1.0 (ref)	2.41 (1.16, 4.99)	2.35 (1.08, 5.12)	3.10 (1.39, 6.90)	0.01	
Menopausal status at blood donation [b]								
Premenopausal	86	161	1.0 (ref)	1.74 (0.54, 5.60)	1.26 (0.42, 3.76)	1.67 (0.57, 4.86)	0.50	0.62
Postmenopausal	143	270	1.0 (ref)	1.27 (0.75, 2.16)	1.07 (0.59, 1.96)	1.62 (0.83, 3.16)	0.26	
Lag time between enrollment and diagnosis [b]								
< 5 years	80	147	1.0 (ref)	0.77 (0.35, 1.69)	1.32 (0.58, 2.99)	1.49 (0.62, 3.58)	0.29	0.46
≥ 5years	150	285	1.0 (ref)	2.02 (1.08, 3.78)	1.05 (0.54, 2.03)	1.70 (0.86, 3.35)	0.42	
Histology [b]								
Serous	120	225	1.0 (ref)	0.86 (0.45, 1.62)	1.04 (0.51, 2.12)	1.26 (0.60, 2.64)	0.44	-
Parity [d]								
Nulliparous	44	70	1.0 (ref)	1.32 (0.32, 5.42)	1.56 (0.44, 5.58)	1.45 (0.49, 5.34)	0.60	0.40
Parous	155	331	1.0 (ref)	1.37 (0.80, 2.32)	0.89 (0.50, 1.59)	1.52 (0.85, 2.72)	0.38	
OC use [d]								
Never	139	237	1.0 (ref)	1.34 (0.74, 2.44)	1.02 (0.55, 1.91)	1.40 (0.74, 2.66)	0.47	0.68
Past	59	131	1.0 (ref)	1.67 (0.68, 4.12)	0.98 (0.40, 2.58)	1.30 (0.48, 3.49)	0.90	

[a] Conditional logistic regression model.

[b] Conditional logistic regression models adjusted for parity (ever/never) and use of oral contraceptives (past/never). Missing data for parity and OC were imputed.

[c] Unconditional logistic regression models adjusted for matching factors (age and menopausal status), parity (ever/never), use of oral contraceptives (past/never), and BMI (log2 continuous scale). Missing data for parity and OC use were imputed.

[d] Unconditional logistic regression models adjusted for matching factors (age and menopausal status), parity (ever/never), and use of oral contraceptives (past/never). Missing data for parity and OC use were imputed.

[e] p-interaction was calculated with prolactin modeled on the continuous scale (after log2 transformation).