Insulin-like Growth Factors and Ovarian Cancer Risk: A Nested Case-Control Study in Three Cohorts

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Abstract

Insulin-like growth factor (IGF)–related hormones and binding proteins (IGFBP) have been implicated in cancer risk. Specifically, two recent, small prospective studies reported a significant positive association between IGF-I and ovarian cancer risk among women diagnosed before age 55 years. Therefore, we examined whether plasma concentrations of IGF-I, IGFBP-3, and IGFBP-2 were associated with risk of epithelial ovarian cancer in a nested case-control study using data from three prospective cohorts: the Nurses' Health Study (NHS), NHSII, and the Women's Health Study (WHS). The present study had 222 cases (159 from NHS/NHSII and 63 from WHS) and 599 controls (matching ratio, 1:3 for NHS/NHSII and 1:2 for WHS). Women ranged in age from 34 to 73 years (mean, 56 years). The relative risk (RR) comparing the top versus bottom quartile of IGF-I was 0.56 (95% confidence interval, 0.32-0.97; P_{trend} = 0.14). The risk did not differ by age at diagnosis (comparable RR for age <55 years at diagnosis, 0.70; RR for age ≥55 years at diagnosis, 0.52). We did not observe any association between IGFBP-3, IGFBP-2, and the ratio of IGF-I to either binding protein and ovarian cancer risk. Overall, our results do not support a positive association between IGF-related proteins and ovarian cancer risk.

Introduction

Insulin-like growth factors (IGF) have been implicated in cancer etiology, including for ovarian cancer (1). Specifically, *in vitro* data support a role of IGF-I in ovarian cancer through increased cell growth (1, 2). Two small prospective studies reported a 2- to 5-fold increased ovarian cancer risk among women <55 years old at diagnosis comparing the top versus bottom tertile of IGF-I levels (3, 4). Paradoxically, retrospective studies report lower IGF-I levels in cases versus controls (5-10).

IGF binding proteins IGFBP-2 and IGFBP-3 may also be important in ovarian cancer etiology (1). IGFBP-3 is the primary binding protein of IGF-I and levels seem to be lower in women with ovarian cancer versus benign lesions (5-8). IGFBP-2 is commonly overexpressed by ovarian cancer cells (11-13), and retrospective studies report higher IGFBP-2 levels in cases versus controls (5, 7, 8). However, prospective studies have not observed an association between these binding proteins and ovarian cancer risk (3, 4, 14).

Given the potential importance of this association, we examined whether plasma concentrations of IGF-I, IGFBP-2, and IGFBP-3 were associated with risk of epithelial ovarian cancer in a prospective, nested case-control study among three cohort studies: the Nurses' Health Study (NHS), NHSII, and the Women's Health Study (WHS).

Materials and Methods

Study Population

NHS/NHSII. The NHS cohort began in 1976 among 121,700 U.S. female registered nurses, ages 30 to 55 years, and the NHSII began in 1989 among 116,609 female registered nurses, ages 25 to 42 years. All women completed an initial questionnaire and have been followed biennially thereafter.

In 1989 to 1990, 32,826 NHS participants (43-69 years old) provided blood samples and completed a short questionnaire (described in ref. 15); follow-up was 98% in 2004. Between 1996 and 1999, 29,611 NHSII participants (32-54 years old) provided blood samples and completed a short questionnaire (described in ref. 16); follow-up was 98% in 2003.

Overall, 161 confirmed epithelial ovarian or peritoneal cancer cases (NHS, 141; NHSII, 20) were diagnosed after blood collection and before June 1, 2004 (NHS) or June 1, 2003 (NHSII; described in ref. 17). Each case was matched to three controls, who had intact ovaries at the time of the case diagnosis, on menopausal status at baseline and diagnosis, age, month of blood collection, time of day of blood draw, fasting status, and postmenopausal hormone use at blood draw.

WHS. The WHS is a completed randomized trial examining low-dose aspirin and vitamin E supplementation for the primary prevention of cancer and cardiovascular disease (18-20). Beginning in 1992, 39,876 female professionals in the United States, >45 years of age and free of cancer (apart from nonmelanoma skin cancer) and cardiovascular disease, were enrolled and completed a baseline questionnaire. Blood samples were collected from 28,345 women before randomization (see ref. 17 for details). This study included women from the treatment and placebo groups (treatment with aspirin or vitamin E was not associated with cancer risk); morbidity and mortality follow-up were 97% and 99%, respectively, in 2004.

Confirmed cases (n = 63) were diagnosed with invasive epithelial ovarian cancer after blood collection and before December 1, 2004. Each case was matched to two controls, who had intact ovaries at the time of the case diagnosis (expect for four cases for whom only one control was identified), on menopausal status at baseline and diagnosis, age, month of blood collection, time since randomization, postmenopausal hormone use at blood collection, and fasting status.

This study was approved by the Committee on the Use of Human Subjects in Research at the Brigham and Women's Hospital.

Menopausal Status. We considered a woman to be premenopausal if (*a*) her periods had not ceased or (*b*) she had a hysterectomy with at least one ovary remaining and was ≤47 (nonsmokers) or ≤45 (smokers) years old (NHS/NHSII) or ≤50 years old (WHS; ref. 21). We considered a woman to be postmenopausal if (*a*) her natural menstrual periods had ceased permanently or (*b*) she had a hysterectomy with at least one ovary remaining and was ≥56 (nonsmokers) or ≥54 (smokers) years old (NHS/NHSII) or ≥60 years old (WHS). The remaining women, most of whom had a simple hysterectomy, were of unknown menopausal status.

Laboratory Assays. Total IGF-I, IGFBP-3, and IGFBP-2 levels were assayed by ELISA after acid extraction, using reagents from Diagnostic Systems Laboratory (Webster, TX), in four batches at the laboratory of one of the authors (M.P.). Case-control sets and samples from the same study were assayed together, ordered randomly, and labeled to mask case-control and quality control status. The intra-assay coefficient of variation from blinded, replicate, quality control samples ranged from 2% to 10%.

Statistical Analysis. The only outliers (22) detected and removed were NHS [IGFBP-3 <2,059 ng/mL (n = 2)] and NHSII [IGF-I <47 ng/mL (n = 1) and IGFBP-3 <2,922 ng/mL (n = 2)], leaving 222 cases and 599 controls for analysis. Of these, 11 were peritoneal and 211 epithelial ovarian, whereas 198 were invasive and 22 borderline. Odds ratios and 95% confidence intervals (95% CI) were determined using conditional logistic regression comparing quartiles of hormones, with cutoff points based on the control distribution within each study. The odds ratio appropriately estimates the relative risk (RR); therefore, we henceforth use the term RR. We also considered age-specific quartiles for analyses stratified by age (<55 versus ≥55 years); however, results did not differ from using the study-specific cutoff points, so only the latter are presented.

To account for potential between-batch variability, we used probit scores for all trend tests. We adjusted for several a priori potential confounders including ever use of postmenopausal hormone (yes, no), parity (continuous), duration of oral contraceptive use (never, <3, 3-<5, 5+ years), simple hysterectomy (yes, no), tubal ligation (yes, no), age at menopause (<45, 45-49, 50+ years, missing/premenopausal), physical activity (<3, 3-<9, 9-<18, 18-<27, ≥27 MET-h/wk), and age at menarche (<12, 12, 13, ≥14 years). Other potential confounders including body mass index and family history of ovarian cancer did not substantially alter the results and therefore were not included in the final model.

In secondary analyses, we excluded cases diagnosed within 2 or 4 years of blood collection and evaluated associations among only epithelial ovarian, invasive, or serous cases. We stratified by age at diagnosis (<55, ≥55 years), menopausal status at blood draw or diagnosis (premenopausal, postmenopausal), body mass index (<25, ≥25 kg/m^2), and postmenopausal hormone use (among postmenopausal women, never/past versus current). These analyses used unconditional logistic regression adjusting for matching factors, including age at blood draw, fasting status, time of blood draw, month of blood draw, postmenopausal hormone use at blood draw, menopausal status at blood and diagnosis, and study.

Results

Women in the NHS/NHSII ranged from 34 to 69 years old (mean, 56 years) and in the WHS from 45 to 73 years old (mean, 56 years) at blood collection (Table 1). In general, characteristics of the participants were similar between the studies. Among premenopausal women at blood collection (n = 62 cases/177 controls), none were current users of oral contraceptives. Further, among postmenopausal women (n = 127 cases/344 controls), 64 cases and 172 controls were using postmenopausal hormone at blood collection. The remaining 33 cases and 78 controls were of unknown menopausal status at blood draw. IGF-I and IGFBP-3 levels were significantly lower in current versus noncurrent postmenopausal hormone users (P < 0.001 for both). Mean IGF-related hormone levels did not differ between cases and controls within the NHS/NHSII or WHS (P ≥ 0.11). The correlation of

Table 1. Characteristics at blood collection of cases and their matched control women from the NHS/NHSII and the WHS

	NHS/NHSII		WHS	
	Cases, mean (SD)	Controls, mean (SD)	Cases, mean (SD)	Controls, mean (SD)
Sample size	159	477	63	122
Age (y)	55.9 (7.9)	55.9 (7.8)	55.7 (7.2)	55.5 (7.0)
Age at menarche (y)	12.5 (1.5)	12.6 (1.4)	12.6 (1.1)	12.5 (1.4)
Parity*	3.2 (1.7)	3.3 (1.5)	2.9 (1.2)	3.0 (1.3)
Body mass index at blood draw (kg/m^2)	25.3 (5.5)	25.1 (4.4)	24.6 (3.8)	25.0 (4.4)
Physical activity (MET-h/wk)	16.9 (17.1)	16.1 (18.0)	14.5 (15.0)	16.7 (16.7)
Premenopausal at blood draw (%)	28.9	29.4	25.4	30.3
Family history of ovarian cancer (%)	9.4	3.6	3.2	5.7
Tubal ligation (%)	14.5	17.2	19.1	27.1
Current use at blood draw of postmenopausal hormones† (%)	43.5	44.0	68.6	73.9
Ever used oral contraceptives (%)	45.9	50.3	65.1	71.3
Median IGF-I‡ (10th-90th percentile; ng/mL)	173 (106-254)	176 (109-284)	168 (109-237)	168 (110-250)
Median IGFBP-3‡ (10th-90th percentile; ng/mL)	4,684 (3,593-6,118)	4,675 (3,607-6,103)	4,029 (3,105-5,070)	4,138 (3,124-5,231)
Median IGFBP-2‡ (10th-90th percentile; ng/mL)	292 (107-641)	287 (108-608)	368 (173-821)	358 (150-803)

*Among parous women only.

† Among postmenopausal women.

‡ In the NHS and NHSII, levels were measured in heparin plasma; in the WHS, levels were measured in EDTA plasma. P values comparing levels between cases and controls in the NHS/NHSII were 0.11 for IGF-I, 0.95 for IGFBP-3, and 0.75 for IGFBP-2. The corresponding P values for WHS were 0.96, 0.93, and 0.66, respectively.

Table 2. RRs (95% CIs) of ovarian cancer by quartile of plasma IGF-related hormone concentrations among women in the NHS/NHSII and the WHS

	Quartile 1	Quartile 2	Quartile 3	Quartile 4	P_{trend}*
IGF-I concentrations (ng/mL)					
NHS/NHSII					
Quartile values (NHS)	<139	139-<169	169-<212	≥212	
Quartile values (NHSII)	<191	191-<247	247-<298	≥298	
n, case/control	50/119	36/119	43/119	30/120	
WHS					
Quartile values	<132	132-<168	168-<219	≥219	
n, case/control	15/30	16/31	19/30	13/31	
Combined †					
Unadjusted ‡	1.0 (reference)	0.73	0.89	0.57	0.12
Adjusted§	1.0 (reference)	0.74 (0.47-1.19)	0.93 (0.58-1.49)	0.56 (0.32-0.97)	0.14
Adjusted + IGFBPs§	1.0 (reference)	0.73 (0.44-1.20)	0.89 (0.51-1.54)	0.51 (0.26-1.01)	0.14
IGFBP-3 concentrations (pg/mL)					
NHS/NHSII					
Quartile values (NHS)	<4,068	4,068-<4,583	4,583-<5,206	≥5,206	
Quartile values (NHSII)	<4,859	4,859-<5,148	5,148-<5,605	≥5,605	
n, case/control	45/118	34/119	38/118	41/120	
WHS					
Quartile values	<3,555	3,555-<4,138	4,138-<4,713	≥4,713	
n, case/control	13/30	19/31	19/30	12/31	
Combined †					
Unadjusted ‡	1.0 (reference)	0.89	0.95	0.89	0.97
Adjusted§	1.0 (reference)	0.85 (0.52-1.36)	0.95 (0.59-1.51)	0.82 (0.49-1.37)	0.74
Adjusted + IGF-I and IGFBP-2§	1.0 (reference)	0.90 (0.54-1.49)	1.10 (0.64-1.90)	1.07 (0.57-2.03)	0.41
IGFBP-2 concentrations (pg/mL)					
NHS/NHSII					
Quartile values (NHS)	<183	183-<288	288-<448	≥448	
Quartile values (NHSII)	<152	152-<263	263-<375	≥375	
n, case/control	42/119	35/119	46/119	36/120	
WHS					
Quartile values	<240	240-<358	358-<531	≥531	
n, case/control	16/30	12/31	19/30	16/31	
Combined †					
Unadjusted ‡	1.0 (reference)	0.81	1.11	0.87	0.52
Adjusted§	1.0 (reference)	0.88 (0.55-1.42)	1.16 (0.73-1.86)	0.89 (0.54-1.46)	0.45
Adjusted + IGF-I and IGFBP-3§	1.0 (reference)	0.90 (0.56-1.44)	1.19 (0.74-1.92)	0.83 (0.50-1.38)	0.58

*Determined using study-specific probit rankings of analyte concentrations.
† $P_{heterogeneity}$ comparing NHS/NHSII versus WHS is 0.85 for IGF-I, 0.77 for IGFBP-3, and 0.96 for IGFBP-2.
‡Calculated using simple conditional logistic regression.
§Adjusted for parity, duration of oral contraceptive use, simple hysterectomy, tubal ligation, ever use of postmenopausal hormones, physical activity, age at menopause, and age at menarche using conditional logistic regression.

IGF-I with age was inverse ($r = -0.41$) and with IGFBP-3 was positive ($r = 0.61$); IGFBP-2 was not correlated with IGF-I ($r = -0.09$) and inversely correlated with IGFBP-3 ($r = -0.24$).

We did not observe significant associations between quartiles of IGFBP-3 (top versus bottom quartile: RR, 0.82; 95% CI, 0.49-1.49; $P_{trend} = 0.74$), IGFBP-2 (RR, 0.89; 95% CI, 0.54-1.46; $P_{trend} = 0.45$; Table 2), or the IGF-I/IGFBP-3 or IGF-I/IGFBP-2 ratios ($P_{trend} = 0.12$ and 0.29, respectively) and ovarian cancer risk. Those in the top versus bottom quartile of IGF-I levels had a reduced risk of ovarian cancer (RR, 0.56;

Table 3. RRs (95% CIs) of ovarian cancer by time of cancer diagnosis and age at diagnosis for IGF-related hormone concentrations among women in the NHS/NHSII and the WHS combined

	n, case/control	Quartile 1	Quartile 2	Quartile 3	Quartile 4	P_{trend}*
Excluding cases diagnosed <2 y after blood draw						
IGF-I	177/599	1.0 (reference)	0.73 (0.44-1.21)	1.01 (0.61-1.65)	0.55 (0.31-0.97)	0.15
IGFBP-3	176/597	1.0 (reference)	0.92 (0.55-1.52)	0.87 (0.52-1.45)	0.87 (0.52-1.44)	0.63
IGFBP-2	177/599	1.0 (reference)	0.94 (0.56-1.59)	1.44 (0.87-2.36)	1.12 (0.67-1.86)	0.13
Age <55 y at diagnosis †						
IGF-I	59/153	1.0 (reference)	1.10 (0.39-3.13)	1.02 (0.36-2.85)	0.70 (0.25-1.97)	0.38
IGFBP-3	59/151	1.0 (reference)	0.80 (0.33-1.93)	0.71 (0.28-1.80)	0.64 (0.25-1.67)	0.35
IGFBP-2	59/153	1.0 (reference)	0.88 (0.37-2.11)	1.17 (0.48-2.84)	0.86 (0.34-2.19)	0.61
Age ≥55 y at diagnosis †						
IGF-I	163/446	1.0 (reference)	0.67 (0.40-1.12)	0.90 (0.54-1.50)	0.52 (0.28-0.95)	0.14
IGFBP-3	162/446	1.0 (reference)	0.86 (0.50-1.47)	0.99 (0.58-1.67)	0.86 (0.50-1.46)	0.83
IGFBP-2	163/446	1.0 (reference)	0.88 (0.51-1.54)	1.22 (0.73-2.05)	0.98 (0.58-1.67)	0.38

NOTE: Data were adjusted for parity, duration of oral contraceptive use, simple hysterectomy, tubal ligation, ever use of postmenopausal hormones, physical activity, age at menopause, and age at menarche, and matching factors including age at blood draw (continuous), fasting status, time of blood draw (1 a.m.-8 a.m., 9 a.m.-noon, 1 p.m.-midnight), month of blood draw (continuous), menopausal status at baseline and diagnosis (premenopausal, postmenopausal, unknown for both), and recent postmenopausal hormone use before blood draw.
*Determined using study-specific probit rankings of hormone concentrations.
† $P_{heterogeneity}$ comparing women diagnosed at age <55 versus ≥55 y compares the slope of the probit rankings of hormone levels and was >0.47 for all hormones.

95% CI, 0.32-0.97); however, the trend test was not statistically significant (P_{trend} = 0.14). Study-specific associations generally were not statistically significant (data not shown); there was no statistical heterogeneity between studies ($P_{heterogeneity}$ = 0.77-0.96). Inclusion of IGF-I, IGFBP-2, and IGFBP-3 in the model simultaneously (Table 2) using age-specific quartile cutoff points or using tertiles (data not shown) did not substantially alter the observed associations.

Results were similar after excluding cases diagnosed within 2 years of blood collection (Table 3). For example, comparing the top versus bottom quartile of IGF-I, the RR was 0.55 (95% CI, 0.31-0.97). Results were similar when excluding 86 cases diagnosed within 4 years after blood collection (data not shown). No statistically significant interactions were observed by age at diagnosis ($P_{heterogeneity} \geq 0.47$), although this may be due to the small number of cases diagnosed before age 55 ($n = 59$). However, IGF-I and IGFBP-3, if anything, seemed to be more inversely associated with ovarian cancer among women diagnosed at older ages, with little or no association in premenopausal women. Results were similar after excluding women taking postmenopausal hormones at blood collection, when examining only invasive epithelial ovarian cancer cases, or when stratifying by menopausal status at diagnosis (data not shown). Results were similar for epithelial ovarian, invasive, or serous cases or when stratifying by fasting status, menopausal status at blood draw, body mass index, or, among postmenopausal women, postmenopausal hormone use (data not shown).

Discussion

In a relatively large prospective, nested case-control study, we did not observe any clear associations between IGF-related hormones and risk of ovarian cancer. We noted a suggestive inverse association between IGF-I and ovarian cancer risk, particularly among older women, but no association among younger women. This is inconsistent with two previous prospective studies, both of which reported an increased risk comparing the top versus bottom tertile of IGF-I levels among women diagnosed before age 55 years, and no association among older women (3, 4). Given that retrospective case-control studies have reported lower IGF-I levels in cases versus controls (5-10), we examined whether our results were influenced by the inclusion of women with possible subclinical disease. However, results were similar after excluding women diagnosed within either 2 or 4 years of blood collection. Differences between the prospective studies may be due to the relatively small sample sizes [n = 132 (ref. 3), 214 (ref. 4), and 222 cases]; however, the study populations have similar ranges of age and follow-up time.

Similar to previous prospective studies (3, 4, 14), we did not observe any clear associations between IGFBP-3 or IGFBP-2 levels and ovarian cancer risk. This is contrary to retrospective case-control studies reporting lower IGFBP-3 and higher IGFBP-2 levels in cases versus controls (5-8). It is probable that the disease process alters circulating levels of these binding proteins, leading to the differences between prospective and retrospective studies.

Contrary to our results, experimental data suggest that IGF-I may increase ovarian cancer risk by stimulating cell growth and invasive potential (1, 2, 11, 13). Therefore, from a biological perspective, it is unlikely that an inverse association exists between IGF-I and ovarian cancer risk. Although the IGF system has autocrine/paracrine effects on the ovary, data do suggest that circulating levels are important in the development of several cancers (23). Our data, when viewed in conjunction with other studies, indicate that these associations should be studied further.

Our study has several limitations. First, the studies collected different sample types (NHS/NHSII used heparin and WHS used EDTA plasma); however, we used study-specific cutoff points to reduce the influence of different blood types on the results. Second, we only had one blood sample per person, which may not reflect exposure over 14 years of follow-up. However, the intraclass correlation over 1 to 3 years was >0.80 for IGF-I and IGFBP-2 and 0.60 to 0.85 for IGFBP-3 (24, 25), suggesting that one sample is fairly representative at least over a 3-year period. The strength of this study was its prospective nature, with blood samples being collected before disease diagnosis, and the number of cases provided reasonable power to detect an association for the primary analyses; we had limited power for subgroup analyses.

Our results were not consistent with a positive association between IGF-I concentrations and risk of ovarian cancer. Further, major binding proteins (IGFBP-3 and IGFBP-2) were not associated with risk. Given the conflicting results of prospective studies, further research should be conducted with larger samples sizes or pooling of existing studies.

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