SERUM COMPONENTS AND RISK OF CANCER - IV

Lack of Association between Risk of Biliary Tract Cancer and Circulating IGF (Insulin-like Growth Factor) -I, IGF-II or IGFBP-3 (IGF-binding Protein 3): A Nested Case-control Study in a Large Scale Cohort Study in Japan (JACC Study)

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Abstract

Biliary tract cancer, encompassing gallbladder and bile duct cancers, has a poor prognosis, but little is known of the etiology. A nested case-control study was here conducted to evaluate the association between serum levels of IGF-I, IGF-II and IGFBP-3 and death from biliary tract cancer. In a large scale cohort study, 35 gallbladder and 42 bile duct cancers were observed during the follow-up. For each subject in the case group, 1-3 control subjects (228 in total) were selected randomly, matched for sex, age (as near as possible) and residential area. The subjects were divided into tertiles by circulating levels of IGF-I, IGF-II or IGFBP-3. Using conditional logistic regression, risks among the tertiles were compared adjusted for defecation, smoking and drinking habits. No remarkable differences in risks of gallbladder or bile duct cancer were observed among tertiles of IGF-I or IGF-II, and no remarkable trend was observed. Circulating IGFBP-3 showed an inverse U-shape association with gallbladder cancer and a U-shaped one with bile duct cancer. Associations between IGF-I or IGF-II and gallbladder or bile duct cancer thus were lacking or very weak. The observed U- and inverse U-shaped association of IGFBP-3 with the cancers is not suggestiveof any meaningful relationships.

Keywords: Japan Collaborative Cohort Study - nested case-control study - biliary tract cancer - IGF

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Introduction

Biliary tract cancer, encompassing gallbladder and bile duct cancers, is the 7th leading cause of cancer death in Japan, and is known to have poor prognosis. Regarding relationships between the cancer risk and lifestyle factors, we have demonstrated importance for constipation, cigarette smoking and drinking habits with regard to gallbladder cancer (Yagyu et al., 2006; 2008), but no risk factors have been established for bile duct cancer. This may be because no lifestyle factors are strongly related.

Many cytokines and growth factors have been identified so far, and associations with several cancers have been shown. If there were any link between a particular growth factor and risk of biliary tract cancer, the factor might be more useful forn prevention of the cancer than non-specific lifestyle factors. Insulin-like growth factors (IGFs) such as IGF-I and IGF-II have roles in the regulation of cell proliferation, differentiation and apoptosis (Yu et al., 2000). The effects of IGFs are regulated by IGF-binding proteins, and IGF-binding protein-3 (IGFBP-3) is the major binding protein in plasma. Recent epidemiological studies have shown that high levels of circulating IGF-I are associated with increased risk of lung, breast, colon, prostate, and pancreatic cancers (Hankinson et al., 1998; Chan et al., 1998; Ma et al., 1999; Yu et al., 1999; McCarty et al., 2001). We have shown that IGF-I and IGFBP-3 are positively associated with risk of pancreatic cancer (Lin et al., 2004).

To test the hypothesis that IGFs may play a role in biliary tract carcinogenesis, a nested case-control study was conducted to evaluate the association between serum levels of IGF-I, IGF-II and IGFBP-3 and cancer death.

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Materials and Methods

The JACC Study (The Japan Collaborative Cohort Study for Evaluation of Cancer Risk Sponsored by the Ministry of Education, Culture, Sports, Science and Technology) was conducted from 1998 to 1990 involving subjects living in 45 municipalities. Details of the cohort design and methods were previously described (Ohno et al., 2001; Tamakoshi A et al., 2005). At baseline in 37 areas, the cohort participants donated blood samples, and their sera were stored in deep freezers at -80ºC until 1999. The proportion of blood donation to the whole JACC Study subjects was approximately 35%. Serum samples of 39,242 subjects aged 40 to 79 years at the baseline, were available for biochemical analysis.

Case ascertainment and control selection

The causes and dates of death among the study subjects were determined by reviewing all death certificates in each study area with the permission of the Director-General of the Prime Minister's Office (Ministry of Public Management, Home Affairs, Post and Telecommunications) till 1997. Participants who had moved out from their study areas at baseline were also identified by reviewing the population-register sheets of cohort members. Those who have died by 1997 from biliary tract cancer were regarded as cases. Using the ICD 10 classification, C23 was defined as gallbladder cancer, and C24 as bile duct cancer. By the end of the period, we identified 77 subjects (35 gallbladder and 42 bile duct cancers). For each case , 1-3 control subjects were selected randomly, matched for gender, age (as near as possible) and residential area. A total of 228 (103 for gallbladder cancer cases and 125 for bile duct cancer cases) subjects formed the control group.

Biochemical assays of sera

Sera were separated from the blood samples as soon

Table 1. Background Data for the Case and Control Subjects

		Biliary tract cancer		Gallbladder cancer		Bile duct cancer	
		Cases	Controls	Cases	Controls	Cases	Controls
Age:	40-49	4	12	3	9	1	3
	50-59	18	52	8	22	10	30
	60-69	32	95	12	36	20	59
	70-79	23	69	12	36	11	33
	Total	77	228	35	103	42	125
	Mean±SD[1]	64.7 ± 7.8	64.8 ± 7.8	64.4 ± 8.7	64.4 ± 8.5	65.0 ± 7.2	65.1 ± 7.1
Male/Female[1]		35/42	104/124	11/24	33/70	24/18	71/54
Smoking habit[2]:	Current	22 (31.4%)	49 (22.8%)	9 (27.3%)	15 (15.2%)	13 (35.1%)	34 (29.3%)
	Ex-smoker	13 (19.2%)	43 (20.0%)	5 (15.2%)	18 (18.2%)	8 (21.6%)	25 (21.6%)
	Never-smoker	35 (50.0%)	123 (57.2%)	19 (57.6%)	66 (66.7%)	16 (43.2%)	57 (49.1%)
Drinking habit[2]:	Current	31 (46.4%)	91 (41.6%)	11 (35.5%)	33 (33.7%)	20 (54.1%)	58 (47.5%)
	Ex-drinker	2 (3.6%)	10 (4.7%)	0 (0.0%)	3 (3.4%)	2 (5.4%)	7 (5.7%)
	Never-drinker	35 (51.5%)	119 (54.1%)	20 (64.5%)	62 (63.3%)	15 (40.5%)	57 (46.7%)
Constipation[2]							
Stool frequency≤4 days		2 (2.7%)	2 (0.9%)	1 (3.3%)	0 (0.0%)	1 (2.4%)	2 (1.6%)
Tendency toward diarrhea: Yes		6 (9.5%)	15 (7.9%)	2 (6.9%)	8 (10.0%)	4 (11.8%)	7 (6.4%)
Serum values (Mean±standard deviation[2])							
IGF-I ng/ml		132.0 ± 60.4	125.7 ± 56.1	132.9 ± 64.2	123.4 ± 63.1	131.2 ± 57.8	127.7 ± 49.8
IGF-II ng/ml		591.6 ± 185.1	579.6 ± 127.7	615.4 ± 186.6	569.4 ± 140.0	571.7 ± 183.8	587.9 ± 116.6
IGFBP-3µg/ml		2.86 ± 1.04	2.87±0.92	2.92 ± 1.20	2.80 ± 1.08	2.81 ± 0.91	2.92 ± 0.77

[1]Matching factor; [2]No significant difference was observed between cases and controls

Table 2. Circulating IGF-I Levels and Risks of Biliary Tract Cancers

				Model 1[1)]		Model 2[2)]		Model 3[3)]	
	Tertile	Range	Case/control	OR	(95%CI)	OR	(95%CI)	OR	(95%CI)
Biliary tract cancer	lowest	≤100	28/77	1.00		1.00		1.00	
	intermediate	>100	21/84	0.68	(0.35-1.32)	0.67	(0.34-1.32)	0.71	(0.33-1.51)
	highest	>140	28/67	1.14	(0.60-2.17)	0.93	(0.47-1.82)	1.08	(0.46-2.54)
	p for trend			0.68		0.85		0.85	
Gallbladder cancer	lowest	≤100	15/42	1.00		1.00		1.00	
	intermediate	>100	5/31	0.46	(0.15-1.44)	0.43	(0.13-1.40)	0.40	(0.11-1.46)
	highest	>140	15/30	1.48	(0.56-3.89)	1.02	(0.36-2.91)	1.13	(0.32-4.03)
	p for trend			0.47		0.99		0.98	
Bile duct cancer	lowest	≤100	13/35	1.00		1.00		1.00	
	intermediate	>100	16/53	0.80	(0.33-1.92)	0.82	(0.33-2.02)	1.02	(0.36-2.92)
	highest	>140	13/37	0.93	(0.37-2.34)	0.90	(0.35-2.32)	1.28	(0.35-4.72)
	p for trend			0.90		0.84		0.71	

[1)]Matched for gender, age and residential area; [2)]Matched for gender, age and residential area, and adjusted for constipation, smoking and drinking habits; [3)]Matched for gender, age and residential area, and adjusted for IGFBP-3, constipation, smoking and drinking habits

as possible at laboratories in each study area and stored at -80˚C until analysis. All the samples were assayed in 1999 and 2000 by trained staff at a single laboratory (the SRL, Inc., Hachioji, Japan) who were blinded to the case/control status of the samples. Serum levels of IGF-I, IGF-II and IGFBP-3 were measured by immunoradiometric assay, using commercially available kits (Daiichi Radioisotope Lab., Tokyo). The ranges of reliable measurement for IGF-I, IGF-II, and IGFBP-3 were 4-2,000 ng/ml, 10-1640 ng/ml, and 0.06-10.10 μg/ml, respectively. The intra- and inter-assay coefficients of variation were 2.15-3.53% and 1.21-4.18% for IGF-I, 2.74-4.45% and 4.23-5.53% for IGF-II 3.16-4.19% and 5.28-8.89% for IGFBP-3, respectively (Ito et al., 2005).

Statistical analysis

The odds ratios and 95% confidence intervals derived from the conditional logistical model were used to examine associations between serum IGFs and biliary tract cancer risk. Calculations were carried out using data sets for gallbladder, bile duct cancer and both combined. For each data set, three models were calculated: adjusted for nothing (matched factors only); constipation, smoking and drinking habits; or IGFBP-3 or IGF-I, constipation, smoking and drinking habits. The cut-off points for the tertiles were determined according to the distribution of serum levels in pooled controls, who were selected as controls for all cancers. The ORs were calculated with the lowest tertile being the reference category. To test for a linear trend across the tertiles, we coded each tertile as 0, 1 or 2 and then incorporated this data into a logistic model as a single variable. All *p*-values were from 2-tailed tests. All analyses were conducted using SAS (SAS institute, Inc., Cary, NC).

Results

Distributions of gender, age, smoking and drinking habits, and constipation are shown in Table 1. No remarkable difference was observed between cases and controls. Associations between serum IGF-I, IGF-II or IGFBP-3 and risk of biliary tract cancer are shown in Tables 2, 3 and 4. No remarkable difference in risks of gallbladder or bile duct cancer was observed among tertiles of IGF-I or IGF-II, and no remarkable trend was observed, either. Regarding IGF-I, odds ratios (ORs, 95 percent confidence intervals: 95% CIs) of intermediate and the highest tertiles compared with the lowest tertile of IGF-I

Table 3. Circulating IGF-II Levels and Risks of Biliary Tract Cancers

				Model 1[1)]		Model 2[2)]		Model 3[3)]	
	Tertile	Range	Case/control	OR	(95%CI)	OR	(95%CI)	OR	(95%CI)
Biliary tract cancer	lowest	≤530	31/80	1.00		1.00		1.00	
	intermediate	>530	22/82	0.69	(0.36-1.30)	0.64	(0.33-1.24)	0.70	(0.31-1.44)
	highest	>630	24/66	0.92	(0.48-1.76)	0.87	(0.44-1.71)	1.16	(0.47-2.82)
	p for trend			0.76		0.62		0.78	
Gallbladder cancer	lowest	≤530	13/39	1.00		1.00		1.00	
	intermediate	>530	11/35	0.94	(0.36-2.43)	0.91	(0.33-2.53)	1.03	(0.33-3.27)
	highest	>630	11/29	1.13	(0.41-3.13)	1.14	(0.37-3.54)	1.21	(0.31-4.70)
	p for trend			0.82		0.84		0.99	
Bile duct cancer	lowest	≤530	18/41	1.00		1.00		1.00	
	intermediate	>530	11/47	0.53	(0.22-1.26)	0.51	(0.21-1.24)	0.54	(0.18-1.58)
	highest	>630	13/37	0.80	(0.34-1.86)	0.71	(0.29-1.74)	1.34	(0.37-4.84)
	p for trend			0.55		0.41		0.72	

[1)]Matched for gender, age and residential area; [2)]Matched for gender, age and residential area, and adjusted for constipation, smoking and drinking habits; [3)]Matched for gender, age and residential area, and adjusted for IGFBP-3, constipation, smoking and drinking habits

Table 4. Circulating IGFBP-3 Levels and Risks of Biliary Tract Cancers

				Model 1[1)]		Model 2[2)]		Model 3[3)]	
	Tertile	Range	Case/control	OR	(95%CI)	OR	(95%CI)	OR	(95%CI)
Biliary tract cancer	lowest	≤2.61	31/94	1.00		1.00		1.00	
	intermediate	>2.61	21/68	0.94	(0.49-1.78)	0.84	(0.43-1.63)	0.79	(0.37-1.68)
	highest	>3.26	25/66	1.15	(0.62-2.14)	1.13	(0.58-2.17)	1.21	(0.53-2.76)
	p for trend			0.69		0.77		0.67	
Gallbladder cancer	lowest	≤2.61	11/52	1.00		1.00		1.00	
	intermediate	>2.61	13/17	3.97	(1.43-11.1)	3.27	(1.11-9.62)	3.66	(1.10-12.1)
	highest	>3.26	11/34	1.64	(0.62-4.36)	1.69	(0.59-4.85)	2.01	(0.55-7.41)
	p for trend			0.41		0.29		0.30	
Bile duct cancer	lowest	≤2.61	20/42	1.00		1.00		1.00	
	intermediate	>2.61	18/51	0.33	(0.13-0.82)	0.32	(0.13-0.83)	0.20	(0.07-0.67)
	highest	>3.26	14/32	0.92	(0.40-2.11)	0.87	(0.36-2.08)	0.79	(0.26-2.44)
	p for trend			0.66		0.62		0.66	

[1)]Matched for gender, age and residential area; [2)]Matched for gender, age and residential area, and adjusted for constipation, smoking and drinking habits; [3)]Matched for gender, age and residential area, and adjusted for IGFBP-3, constipation, smoking and drinking habits

and p-value for trend were 0.40 (0.11-1.46), 1.13 (0.32-4.03) and 0.98, respectively for gallbladder cancer, and 1.02 (0.36-2.92), 1.28 (0.35-4.72) and 0.71, respectively for bile duct cancer. Regarding IGF-II, they were 1.03 (0.33-3.27), 1.21 (0.31-4.70) and 0.99, respectively for gallbladder cancer, and 0.54 (0.18-1.58), 1.37 (0.37-4.84) and 0.72, respectively for bile duct cancer.

Among tertiles of IGFBP-3, the intermediate tertile showed an elevated risk of gallbladder cancer (OR (95% CI) was 3.66 (1.10-12.12)), and reduced risk of bile duct cancer (OR (95% CI) was 0.20 (0.07-0.67)) compared with the lowest tertile. The significance remained after adjustment for IGF-I, constipation, smoking and drinking habits. The highest tertiles showed risks between the lowest and the intermediate tertiles of both gallbladder (OR (95% CI) was 2.01 (0.55-7.41)) and bile duct cancers (OR (95% CI) was 0.79 (0.26-2.44)). No significant trend was observed (p-values for trend were 0.30 and 0.66, respectively). Results were similar for IGFBP-3 in both gallbladder and bile duct cancers, when the estimate was further adjusted for IGF-II, constipation, smoking and drinking habits (data not shown).

Discussion

As far as we are aware, no previous studies have evaluated the relationships between circulating IGF-I, IGF-II or IGFBP-3 and risk of biliary tract cancer. This nested case-control study may be thus the first with this purpose. No remarkable association or trend was observed between IGF-I or IGF-II and gallbladder or bile duct cancer. Either there is no association, or if one exists it is very weak. In this aspect, biliary tract cancer may be different from pancreatic (McCarty et al., 2001; Lin et al., 2004), colon (Chanet al., 1998), lung (Yu et al, 1999), breast (Hankinson et al., 1998; Vatten et al., 2008) and prostate (Ma et al., 1999; Roddam et al., 2008) cancers, the risks which are positively related with circulating IGF-I, but may be similar with stomach cancer (Pham et al., 2007). Studies with larger sample sizes may be necessary to conclusively preclude an associations .

Circulating IGFBP-3 showed an inverse U-shape association with gallbladder cancer and a U-shape one with bile duct cancer. It seems difficult to explain the mechanism of U- or inverse U-shaped associations. If real relationships exist, IGF-I and IGF-II would also be expected to have shown some associations with the risk. The observed data do not suggest any meaningful relationships, although further studies may be needed to detail the effects of circulating IGFBP-3. From the results of this study, gallbladder and bile duct cancers may differ in this regard and therefore should be analyzed separately at least when relationships with IGFs are explored.

One of limitations of this study was that the endpoint was not incidence but death. However, as biliary tract cancers are known to have a poor prognosis, little difference in results is expected between endpoints of death and incidence. Another limitation is the relatively small sample size. However, as the cases and controls were from the same cohort, biases expected from the small sample size may not be so large.

In conclusion, this nested case-control study showed no clear association between circulating IGF-I, IGF-II or IGFBP-3 and gallbladder or bile duct cancer.

Member List of the JACC Study Group

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