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A prospective study of autoantibodies to Ezrin and pancreatic cancer risk

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Abstract

Purpose—No biomarker is available for pancreatic cancer early detection, but a small prospective European study involving 16 cases and 32 controls raised the possibility that anti-Ezrin autoantibodies may be associated with risk of pancreatic cancer. We aimed to validate this finding in a case-control study nested within a prospective study in the United States.

Methods—Levels of anti-Ezrin autoantibodies were examined using ELISA in pre-diagnostic plasma samples of 73 cases and 145 matched controls. Paired t-test and paired signed rank tests were used to determine the difference between two groups and conditional logistic regression was used to evaluate the association between anti-Ezrin autoantibody levels and risk of developing pancreatic cancer.

Results—No association was found between levels of anti-Ezrin plasma autoantibodies and subsequent risk of developing pancreatic cancer.

Conclusion—Anti-Ezrin autoantibodies did not appear to be useful as a plasma biomarker for early detection of pancreatic cancer.

Keywords

Ezrin; autoantibodies; pancreatic cancer risk; prospective study

Introduction

Pancreatic cancer is the fourth most common cause of cancer death in United States (1). The 5-year relative survival rate is only 7.2% (2). More than half (53%) of patients are diagnosed

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No potential conflicts of interest were disclosed.

at a distant stage, at which point 5-year survival is less than 3% (2). Later-stage diagnosis is due not only to an absence of clinical symptoms but also to a lack of reliable detection methods for screening or diagnosing pancreatic cancer in early stages when surgical removal of the tumor is still possible. Early detection is the key strategy to reducing pancreatic cancer mortality.

Ezrin is a member of the ezrin-radixin-moesin family and works downstream of cell-surface receptors through the activation of Rho and PI3K/Akt signaling pathways (3). Ezrin is overexpressed in many cancers, including pancreatic ductal adenocarcinoma (PDAC) and pancreatic intraepithelial neoplasia lesions (4). A recent study of 16 PDAC cases and 32-matched controls from the European Prospective Investigation into Cancer and Nutrition (EPIC) study, found that levels of anti-Ezrin autoantibodies were significantly higher in pre-diagnostic serum samples from PDAC cases compared to matched controls (5). We sought to validate this finding in a case-control study nested within the prospective Southern Community Cohort Study (SCCS).

Materials and Methods

Study population

Details of the SCCS have been described elsewhere (6;7). Briefly, between 2002 and 2009, nearly 73,500 residents of 12 southern states were enrolled in the cohort at community health centers (CHCs), 53% of whom provided a blood sample upon their entry into the cohort. The blood samples were collected in a 10-ml EDTA tube and a 10-ml serum tube. The vast majority (>95%) of blood samples were processed within 24 hours of collection and stored at -80°C . The plasma samples were used for the assays of anti-Ezrin autoantibodies.

Case identification and control selection

Incident pancreatic cancer cases were identified through linkage with state cancer registries and/or from National Death Index mortality records. Included in the current study are 73 cases and 145 controls individually matched to cases in a 2:1 ratio on age (± 2 years), race (African American, European American, or other), and sex, as well as date (± 6 months) and site (CHC) of study enrollment.

Measurement of blood levels of anti-Ezrin autoantibodies

Human circulating anti-Ezrin autoantibodies were measured by enzyme-linked immunosorbent assay (ELISA) following the method described by Capello et al. (5). Briefly, 100 μl of 1 to 200 diluted plasma samples were incubated in microtitration wells pre-coated with the recombinant protein of *Homo sapiens* Ezrin, transcript variant 1 (OriGene Technologies). After washing, HRP-conjugated rabbit anti-human IgG (dilution 1:1000; Santa Cruz Biotechnology) was added to the wells and incubated. Following another washing, the immobilized autoantibodies were reacted with TMB Substrates. The absorbance was measured at 450 nm. All samples were assayed in duplicate and the results represent mean values. All samples were measured in the same batch, and samples from the matched case-control sets were analyzed in the same assay plate. Laboratory staff members

were blinded to the case-control status of samples and the identity of quality control samples included in the study.

Statistical analysis

Case-control differences for selected baseline characteristics were assessed using a chi-square test. The data were skewed and thus median and geometric means were estimated. Paired t-tests and paired signed rank tests were applied to compare anti-Ezrin autoantibody levels between cases and controls. The association between anti-Ezrin autoantibody levels and risk of pancreatic cancer was analyzed using conditional logistic regression, conditioned on matching variables and additionally adjusted for history of diabetes. *P* values were based on two-sided tests.

Results

The baseline characteristics of all participants are shown in Table 1. Except that cases were marginally more likely to have diabetes ($P = 0.06$), cases and controls were similar in terms of BMI, smoking status, and alcohol intake. Anti-Ezrin autoantibody levels were similar among participants from different recruitment sites, and were inversely correlated with age in cases ($P = 0.03$), but not in controls ($P = 0.14$).

No case-control difference was observed in geometric mean or median of plasma anti-Ezrin autoantibody levels (Table 2). Additional analyses stratified by the median interval between blood collection and diagnosis of pancreatic cancer (i.e., 48 months) also showed no case-control differences (Table 2).

Plasma anti-Ezrin autoantibody level was not associated with pancreatic cancer risk. Compared to the lower tertile, the odds ratios (ORs) of developing pancreatic cancer are 1.07 (95% confidence interval (CI): 0.52–2.19) and 1.15 (95% CI: 0.57–2.33) for the middle and upper tertiles, respectively ($P_{\text{for trend}} = 0.70$), after being adjusted for age and diabetes history (Table 3). A null association was found both for cancer diagnosed within 48 months and for cancer diagnosed at more than 48 months after blood collection (Table 3). Analyses stratified by race (African American or European American) revealed null results as well (data not shown).

Discussion

Autoantibodies to tumor-associated antigens may arise before and during tumor formation, making them possible biomarkers for early detection of cancer (8), including pancreatic cancer (9). To identify tumor antigens associated with early PDAC development, Capello et al. (5) conducted experiments in genetically engineered mice that spontaneously developed PDAC. A common mouse-to-human autoantibody signature, directed against six antigens, was found. Of the six antigens, Ezrin displayed the highest frequency of autoantibodies in the mice with early disease and in PDAC patients with resectable disease. Serum reactivity against whole protein extracts of the CF-PAC-1 human PDAC cell line showed that serum from PDAC patients had a much higher frequency of autoantibodies to Ezrin than healthy subjects (56% vs. 10%, $P < 0.0001$) (5). Among the 16 PDAC cases and 32-matched

controls from the prospective EPIC study, levels of anti-Ezrin autoantibodies were significantly higher in pre-diagnostic serum samples from PDAC cases compared to matched controls (5). In our study conducted in the prospective SCCS, including 73 pancreatic cancer cases and 145 controls, we found no evidence that plasma levels of anti-Ezrin autoantibodies were related to subsequent development of pancreatic cancer.

Ezrin plays an important role in cell adhesion and migration and has been linked to tumor metastasis (3;10). It has been reported that Ezrin is overexpressed in many cancers, including PDAC and pancreatic intraepithelial neoplasia lesions (4). Piao et al. (11) recently evaluated the association of tumor Ezrin protein level with progression among 106 PDAC patients. High expression of Ezrin in tumor tissue was a significant independent poor prognostic factor in PDAC with an adjusted HR of 2.16 (95% CI: 1.38-3.39) (11). A recent meta-analysis including 6,675 cancer patients from 55 studies showed that elevated Ezrin expression was associated with a worse prognosis in patients with cancers, with the pooled HRs of 1.86 (95% CI: 1.51–2.31) for overall survival, 2.55 (95% CI: 2.14–3.05) for disease-specific survival, and 2.02 (95% CI: 1.13– 3.63) for disease-specific survival/metastasis-free survival (12).

Our study included both African and European Americans, and the null association was observed in both populations. Our study had a shorter median follow-up time (48 months) than the Capello et al. study (63 months). Although our study included only 73 pancreatic cancer cases and 145 controls, it has 99% statistical power to detect a one standard deviation mean difference between cases and controls.

In summary, results of our study do not support anti-Ezrin autoantibodies as a useful plasma biomarker for the early detection of pancreatic cancer.

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Table 1

Baseline characteristics of pancreatic cancer cases and matched controls, SCCS

Baseline characteristics	Cases (n=73)	Controls (n=145)	P
Sex [n (%)]			
Male	41 (56.16)	82 (56.55)	0.96
Female	32 (43.84)	63 (43.45)	
Race [n (%)]			
European American	15 (21.43)	35 (24.31)	0.64
African American	55 (78.57)	109 (75.69)	
Age at blood collection [n (%)]			
<60 years	45 (61.64)	91 (62.76)	0.98
60-69 years	19 (26.03)	36 (24.83)	
70 years	9 (12.33)	18 (12.41)	
Education [n (%)]			
< High school	25 (34.25)	57 (39.31)	0.73
High school	21 (28.77)	36 (24.83)	
> High school	27 (36.99)	52 (35.86)	
Income [n (%)]			
< \$15,000/year	42 (58.33)	88 (61.97)	0.54
\$15,000-24,999	22 (30.56)	34 (23.94)	
\$25,000	8 (11.11)	20 (14.08)	
BMI [n (%)]			
<25	22 (30.56)	32 (22.07)	0.30
25-29.99	24 (33.33)	47 (32.41)	
30	26 (36.11)	66 (45.52)	
Smoking status [n (%)]			
Current	39 (53.42)	67 (46.21)	0.57
Former	14 (19.18)	35 (24.14)	
Never	20 (27.40)	43 (29.66)	
Alcohol, Unit Per Day [n (%)]			
<0.7254	53 (73.61)	109 (75.69)	0.67
0.7254-3.9617	13 (18.06)	20 (13.79)	
3.9617	6 (8.33)	15 (10.42)	
Family history of cancer [n (%)]			
No	33 (45.21)	78 (53.79)	0.23
Yes	40 (54.79)	67 (46.21)	
History of diabetes [n (%)]			
No	50 (68.49)	116 (80.00)	0.06
Yes	23 (31.51)	29 (20.00)	

Table 2

Baseline anti-Ezrin autoantibody levels among pancreatic cancer cases and matched controls, SCCS

All cases			
	Cases (n=73)	Controls (n=145)	P
Geometric mean (95% CI)	0.67 (0.59-0.77)	0.64 (0.58- 0.70)	0.40 ^a
Median (25 percentile-75 percentile)	0.58 (0.42-0.94)	0.63 (0.43-0.90)	0.55 ^b
Follow-up time 48 months			
	Cases (n=37)	Controls (n=73)	P
Geometric mean (95% CI)	0.59 (0.50-0.71)	0.61 (0.54- 0.69)	0.98 ^a
Median (25 percentile-75 percentile)	0.58 (0.42-0.74)	0.60 (0.42-0.87)	0.75 ^b
Follow-up time > 48 months			
	Cases (n=36)	Controls (n=72)	P
Geometric mean (95% CI)	0.76 (0.62-0.92)	0.67 (0.58- 0.76)	0.27 ^a
Median (25 percentile-75 percentile)	0.61 (0.50-1.15)	0.65 (0.44-0.97)	0.26 ^b

^aP values were derived from paired t-tests for case-control differences^bP values were derived from paired signed rank tests for case-control differences

Table 3Association of baseline anti-Ezrin autoantibody levels and risk of pancreatic cancer^a

Anti-Ezrin autoantibody levels ^b	Cases	Controls	OR (95%CI)
All participants			
<0.495	23	50	1.0 (reference)
0.495-0.767	24	48	1.07 (0.52-2.19)
>0.767	26	47	1.15 (0.57-2.33)
P for trend			0.70
Follow-up time 48 months			
<0.495	14	27	1.0 (reference)
0.495-0.767	14	26	1.01 (0.38-2.65)
>0.767	9	20	0.85 (0.31-2.35)
P for trend			0.75
Follow-up time > 48 months			
<0.495	9	23	1.0 (reference)
0.495-0.767	10	22	1.27 (0.38-3.33)
>0.767	17	27	1.46 (0.53-4.04)
P for trend			0.45

^aConditional logistic regression conditioned on age, race, sex, as well as date and site of study enrollment, additionally adjustment for diabetes history.

^bO.D. values from ELISA assays