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Original Article

Fish consumption doesn't reduce the risk of hepatocellular carcinoma

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Abstract: Background: Several observational studies have investigated the association between fish consumption and the risk of hepatocellular carcinoma (HCC), however, the results were inconsistent. Hence, we performed a meta-analysis of observational studies to evaluate the effect of fish consumption on HCC risk. Methods: A systematic search was performed using the Pubmed, Embase, and Cochrane Library Central database for case-control and coshort studies that assessed fish intake and HCC risk. Fixed-effect and random-effect models were used to estimate summary relative risks (RR) and the corresponding 95% confidence intervals (CIs). Subgroup analyses and sensitivity analysis were also performed. Results: Nine case-control studies and three cohort studies were included, involving a total of 1,071,458 participants and 2,627 HCC cases. Meta-analysis showed that there was no association between fish consumption and a significant reduction in HCC incidence (RR = 0.85, 95% CI [0.62, 1.17]). In our subgroup analyses, the result was substantially affected by adjustment for hepatic viruses' infection status. Sensitivity analysis confirmed the stability of results. Furthermore, there was no evidence of publication bias as suggested by Begg's *P* value (P = 0.411) and Egger's (P = 0.596) test. Conclusions: In conclusion, our results do not support a significant inverse association of fish consumption with HCC risk. More in-depth studies are warranted to report more detailed results, including stratified results by fish types, preparation methods, and gender.

Keywords: Fish, hepatocellular carcinoma, dietary, epidemiology, meta-analysis

Introduction

Liver cancer is the sixth most commonly diagnosed cancer and the third most common cause of cancer-related deaths worldwide [1]. In 2008, about 750,000 liver cancers were reported worldwide, with about 700,000 deaths [2, 3]. Hepatocellular carcinoma (HCC) is the main type of liver cancer, accounting for over 90% of cases worldwide [3]. The established risk factors of HCC are hepatitis B and C (HBV/HCV) virus infection, aflatoxin exposure, tobacco smoking, and alcohol abuse accompanied by liver cirrhosis [4-10]. Although diet is an important factor in the development of HCC, the role of diet in the etiology of HCC remains unclear, except with regard to alcohol consumption and aflatoxin contamination. Fish is a rich source of n-3 polyunsaturated fatty acids (PUFAs), such as eicosapentaenoic acid (EPA), docosapentaenoic acid (DPA), and docosahexaenoic acid (DHA) [11-13]. Previous studies have shown that fish consumption was linked favorably or unfavorably to the risk of several cancers [14-21]. There are several case-control and prospective cohort studies investigating the association between fish intake and HCC risk, however, the results were inconsistent. To our knowledge, there has been no comprehensive quantitative assessment of the association between fish intake and HCC incidence. Hence, we performed a meta-analysis of observational studies to evaluate the effect of fish consumption on the risk of developing HCC.

Methods

Study identification

This meta-analysis was conducted following the Preferred Reporting Items for Systematic reviews and Meta-Analyses guidelines (PRISMA) [22], and the meta-analysis of observational studies in epidemiology (MOOSE) guidelines [23]. A literature search was carried out using Pubmed (1966 to May 2013), Embase (1947 to

May 2013), and Cochrane Library Central database (1967 to May 2013). There were no restriction of origin and languages. Search terms included: "fish" or "seafood" and "cancer" or "neoplasm" or "malignancy" and "hepatocellular" or "liver". The reference lists of each comparative study included in this meta-analysis and previous reviews were manually examined to identify additional relevant studies.

Study selection

Two reviewers independently selected eligible case-control and cohort studies that investigated fish intake and HCC risk. Disagreement between the two reviewers was settled by discussing with the third reviewer. Inclusion criteria were: (i) used a case-control or cohort study design; (ii) evaluated the association between fish intake and HCC risk; (iii) presented odds ratio (OR), relative risk (RR), or hazard ratio (HR) estimates with its 95% confidence interval (CI). Exclusion criteria: (i) the study was review article, case report, letter to the editor or editor comment; (ii) have no available data; (iii) follow-up less than one year. When there were multiple publications from the same population, only data from the most recent report were included in the meta-analysis and the remaining were excluded. Studies reporting different measures of RR like risk ratio, rate ratio, hazard ratio, and odds ratio were included in the meta-analysis. In practice, these measures of effect yield a similar estimate of RR, since the absolute risk of HCC is low.

Data extraction

The following data was collected by two reviewers independently using a purpose-designed form: name of first author, publishing time, country of the population studied, study design, study period, number of cancer cases and subjects, dietary assessment method, type of fish, quantity of intake, the study-specific adjusted ORs, RRs, or HRs with their 95% CIs for the highest category of fish consumption versus the lowest, confounding factors for matching or adjustments.

Methodological quality assessment

We used Newcastle-Ottawa scale to assess the methodologic quality of cohort and case-control studies. The Newcastle-Ottawa Scale contains eight items that are categorized three categories: selection (four items, one star each), comparability (one item, up to two stars), and exposure/outcome (three items, one star each). A "star" presents a "high-quality" choice of individual study. With consideration that there is a correlation between caloric intake and nutrient consumption, and possibly a direct or indirect causal relation between caloric intake and HCC risk, the scoring system was modified by adding an item in which a study with data analysis that used an energy-adjusted residual or nutrient-density model received an additional Star [24]. Hence, the full score was 10 stars, and the high-quality study was defined as a study with ≥ 7 awarded stars.

Data synthesis and analysis

Heterogeneity was assessed using the Cochran Q and I^2 statistics. For the Q statistic, a *P* value < 0.10 was considered statistically significant for heterogeneity; for the I^2 statistic, heterogeneity was interpreted as absent (I^2: 0%-25%), low (I^2: 25.1%-50%), moderate (I^2: 50.1%-75%), or high (I^2: 75.1%-100%) [25]. Some studies presented individual risk estimates according to the different types of fish and did not report the effect of total fish consumption. In this situation, the study-specific effect size in overall analysis was calculated by pooling the risk estimates of the various fish types, using the inverse-variance method. Subgroup analyses were carried out according to (i) study quality, (ii) study design (cohort versus case-control studies), (iii) geographic location (Europe versus Asia versus North America), (iv) number of adjustment factors ($n \geq 7$ versus $n \leq 6$), adjustment for hepatitis viruses infection (yes, no), adjustment for alcohol intake (yes, no), adjustment for smoking status (yes, no), adjustment for body mass index (BMI) (yes, no). Pooled RR estimates and corresponding 95% CIs were calculated using the inverse variance method. When substantial heterogeneity was detected($I^2 \geq 50\%$), the summary estimate based on the random-effect model (DerSimonian-Laird method) [26] was reported, which assumes that the studies included in the meta-analysis had varying effect sizes. Otherwise, the summary estimate based on the fixed-effect model (the inverse variance method) [27] was reported, which assumes that the studies included in the meta-analysis had the same effect size. We carried out sensitivity analyses by excluding one study at a time to explore whether the

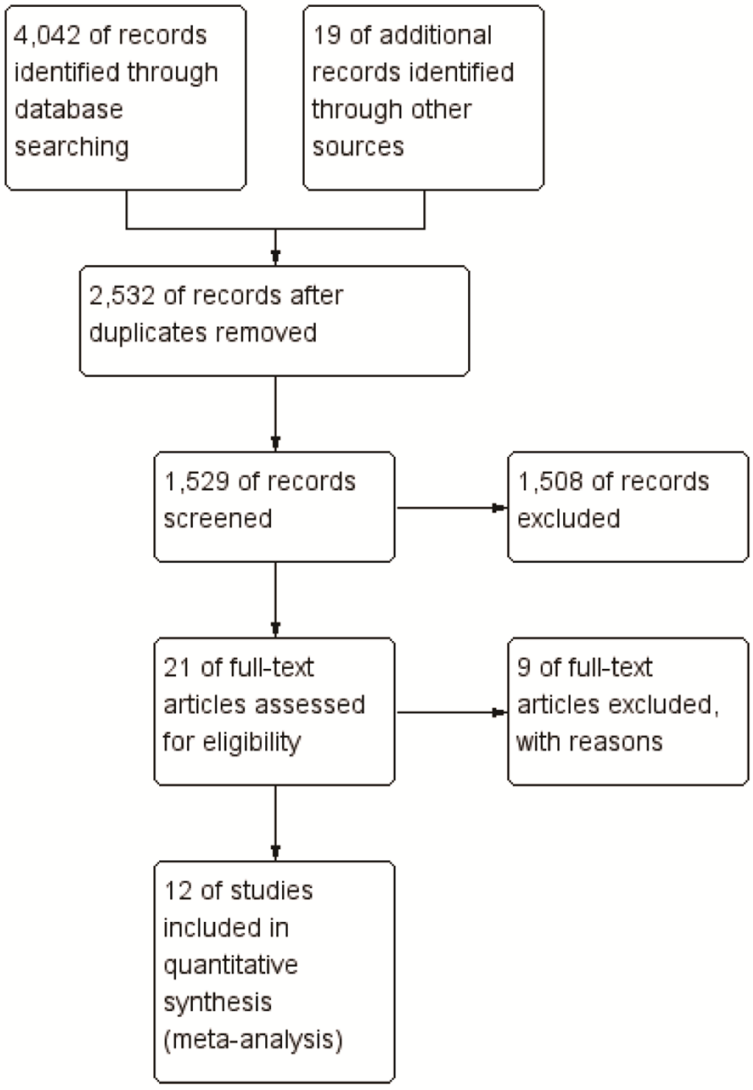

Figure 1. Flow diagram of screened, excluded, and analyzed publications.

results were strongly influenced by a specific study. To better investigate the possible sources of between-study heterogeneity, a meta-regression analysis was performed [28]. Publication bias was assessed using Begg and Mazumdar adjusted rank correlation test and the Egger regression asymmetry test [29, 30]. All analyses were performed using Stata version 11.0 (StataCorp, College Station, TX).

Results

Literature search and study characteristics

The results of the literature search are shown in **Figure 1**. A total of 4,061 citations were identified during the initial search. On the basis of the title and abstract, we identified 21 papers. After detailed evaluation, nine studies were excluded. At last, the remaining 12 studies published between 1982 and 2013 were included in the meta-analysis, involving a total of 2,627 HCC cases and 1,071,458 participants. Of these 12 studies, one was population-based case-control studies [31], eight were hospital-based case-control studies [32-39], and the remaining three were cohort studies [40-42]. Six studies were conducted in Asia [31, 35-37, 39, 41], five in Europe [32-34, 38, 40], and the remaining one in North America [42]. Most studies used food frequency questionnaires (FFQ) for the assessment of fish consumption (Baseline data and other details are shown in **Table 1**). Table S1 summarizes the quality scores of cohort studies and case-control studies. The Newcastle-Ottawa Scale scores for the included studies ranged from 5 to 10, with a median 7.5. The median scores of cohort studies and case-control studies were 9 and 7, respectively. 8 studies (66.6%) were deemed to be of a high quality (≥ 7).

Main analysis

Because of significant heterogeneity (I^2 = 75.1%, P < 0.001) was observed, a random-effects model was chosen over a fixed-effects model and we found that there was no association between fish consumption and a significant reduction in HCC incidence (RR = 0.85, 95% CI [0.62, 1.17]). Both multivariable adjusted RR estimates with 95% CIs of each study and combined RR is shown in **Figure 2**.

Subgroup analyses and sensitivity analysis

We found a significant association between fish intake and decreased HCC risk among cohort studies (RR = 0.74, 95% CI [0.55, 0.98]), however, there was no significant association

Table 1. Characteristics of studies included in the present meta-analysis

Author	Publication year	Country	Study design	Study period	Sex	Methods used for dietary assessment	Cases/ Subjects	Type of fish	Units and comparison groups	Confounders for adjustment
Fedirko V	2013	Western Europe	cohort study	1992-2010	M/F	country-specific, validated dietary questionnaires	191/477,206	Total fish	Q4 vs Q1	smoking status, physical activity level, diabetes status, lifetime alcohol intake pattern, BMI, baseline intakes of coffee, alcohol, meat, and dietary fiber
Sawada N	2012	Japan	cohort study	1990-1994	M/F	FFQ 138 items	398/90,296	Total fish	High vs low	age, area, sex, HCV, HBsAg, ALT level, smoking status, alcohol frequency, BMI, past history of diabetes mellitus, and intake of coffee, soy foods, vegetables, vegetable oil, protein, and iron
Daniel CR	2011	USA	cohort study	1995–1996	M/F	FFQ 124 items	582/492,186	Total fish	Q5 vs Q1	meat intake, age, sex, education, marital status, family history of cancer, race, BMI, smoking status, frequency of vigorous physical activity, menopausal hormone therapy in women, intake of alcohol, fruit, vegetables, and total energy
Kanazir M	2010	Serbia	hospital based case-control study	2004-2007	M/F	standard questionnaire 13 main food groups or food items	45/135	Total fish	Weekly vs rarely	gender and age
Talamini R	2006	Italy	hospital based case-control study	1999–2002	M/F	FFQ 63 items	185/597	Total fish	4Q vs 1Q	gender, age, centre, education, place of birth, drinking habits, maximal lifetime alcohol intake, hepatitis viruses, and total energy intake
Yu S	2002	China	population based case-control study	1995-1997	M/F	interviewer-administered questionnaire	248/496	Salted fish, fresh fish	High vs low	age, sex, residence, and HBV
Fernandez E	1999	Italy	hospital based case-control study	1983-1996	M/F	FFQ 37 items	428/8,418	Total fish	Servings/week ≥ 2 vs < 1	age, sex, area of residence, education, smoking, alcohol consumption, and BMI
Wang ZJ	1996	China	hospital based case-control study	1994-1995	M/F	FFQ 91 items	96/240	Salted fish, fresh fish	High vs low	age, sex, HCV, HBsAg, occupation, education, income, alcohol consumption, history of liver disease, history of hepatitis, pickles intake
Srivatanakul P	1991	Thailand	hospital based case-control study	1987-1988	M/F	FFQ 11 items	65/130	Fermented fish	Servings/day ≥ 3 vs < 3	age and educational level
Lu SN	1988	China	hospital based case-control study	1985	M/F	standardized questionnaire	131/338	Raw fish	Occasional vs never	age, sex and hepatitis B surface antigen
La Vecchia C	1988	Italy	hospital based case-control study	1984-1987	M/F	FFQ 14 items	151/1,202	Total fish	High vs low	none
Lam KC	1982	China	hospital based case-control study	1977-1980	M/F	FFQ 14 items	107/214	Salted fish	Daily vs less than weekly	none

M: male; F: female; BMI: body mass index; FFQ = food frequency questionnaire.

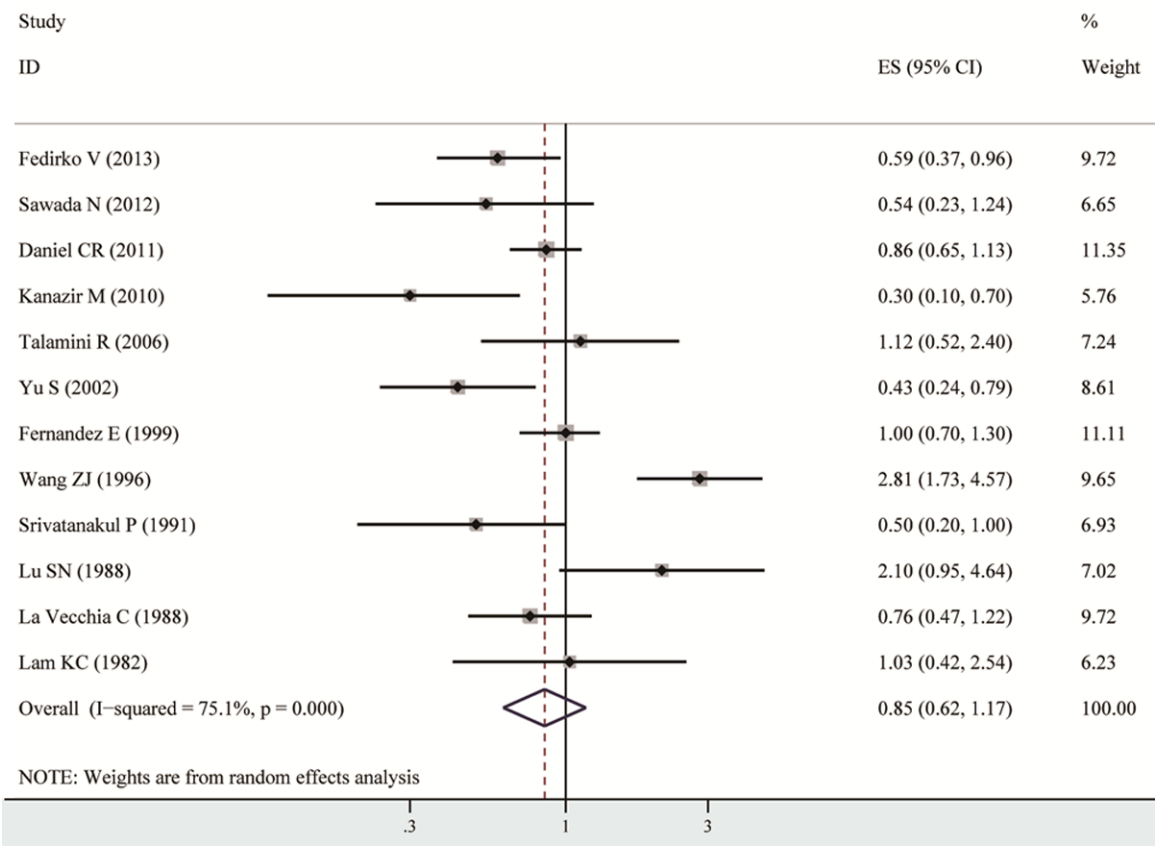

Figure 2. Forest plot: overall meta-analysis of fish consumption and HCC risk. Squares indicated study-specific risk estimates (size of square reflects the study-statistical weight, i.e. inverse of variance); horizontal lines indicate 95% confidence intervals; diamond indicates summary relative risk estimate with its corresponding 95% confidence interval.

between fish intake and HCC risk among case-control studies (RR = 0.92, 95% CI [0.59, 1.43]), presented in **Table 2**. When stratified the various studies by geographic location, no significant association was noted among studies conducted in Europe (RR = 0.75, 95% CI [0.53, 1.06]), Asian (RR = 0.96, 95% CI [0.46, 2.01]), and North America (RR = 0.86, 95% CI [0.65, 1.13]). When we examined whether the associations differed by adjustment for BMI, alcohol intake, and smoking status, the associations did not vary by these factors (shown in **Table 2**). However, the associations varied significantly by adjustment for hepatitis viruses infection status. No significant association was noted among studies which adjusted for hepatitis viruses infection status (RR = 1.11, 95% CI [0.50, 2.46]), while a significant inverse association was found among studies which haven't adjusted for hepatitis viruses infection status (RR = 0.76, 95% CI [0.60, 0.96]). Further, it was observed that studies with higher control for potential confounders ($n \geq 7$) as well as studies with lower control ($n \leq 6$) presented no significant association between fish intake and reduced HCC risk (RR = 1.00, 95% CI [0.66, 1.52] and RR = 0.69, 95% CI [0.42, 1.14], respectively) (shown in **Table 2**). When we did subgroup analysis of high-quality studies, we found there was no significant association between fish intake and reduced HCC risk (RR = 0.84, 95% CI [0.63, 1.13]). To test the robustness of association and characterize possible sources of statistical heterogeneity, sensitivity analyses were carried out by excluding studies one-by-one and analyzing the homogeneity and effect size for all of rest studies. Sensitivity analysis indicated that no significant variation in combined RR by excluding any of the study, confirming the stability of present results.

Meta-regression analysis

To better investigate the possible sources of between-study heterogeneity, a meta-regression analysis was performed. Study design,

Table 2. Subgroup analysis of fish intake and the risk of hepatocellular carcinoma

	No. of studies	Pooled estimate		Tests of heterogeneity	
		RR	95% CI	*P* value	I^2 (%)
All studies	12	0.85	0.62-1.17	< 0.001	75.10
High-quality studies (scores ≥ 7)	8	0.84	0.63-1.13	0.11	44.60
Study design					
Cohort	3	0.74	0.55-0.98	0.29	19.50
Case-control	9	0.92	0.59-1.43	< 0.001	79.20
Geographic location					
Europe	5	0.75	0.53-1.06	0.08	51.50
Asia	6	0.96	0.46-2.01	< 0.001	84.70
North America	1	0.86	0.65-1.13	/	/
Adjusted for confounders					
Number of adjustment factors					
n ≥ 7 confounders	6	1.00	0.66-1.52	< 0.001	80.40
n ≤ 6 confounders	6	0.69	0.42-1.14	0.01	64.80
Major confounders adjusted					
BMI					
Yes	4	0.81	0.64-1.04	0.22	32.00
No	8	0.90	0.51-1.58	< 0.001	81.80
Alcohol					
Yes	6	1.00	0.66-1.52	< 0.001	80.40
No	6	0.69	042-1.14	0.01	64.80
Smoking status					
Yes	4	0.81	0.64-1.04	0.22	32.00
No	8	0.90	0.51-1.58	< 0.001	81.80
Hepatitis viruses infection					
Yes	5	1.11	0.50-2.46	< 0.001	85.80
No	7	0.76	0.60-0.96	0.15	36.10
Processed fish	4	0.94	0.46-1.93	0.03	67.50

RR = relative risks; CI = confidence intervals.

geographic location, number of adjustment factors, adjustment for hepatitis viruses infection, adjustment for alcohol intake, adjustment for smoking status, adjustment for BMI, which may be potential sources of heterogeneity, were tested by a meta-regression method. Meta-regression analysis revealed that study design (P = 0.039) was the source of heterogeneity.

Publication bias

In the present meta-analysis, no publication bias was observed among studies using Begg's *P* value (P = 0.411); Egger's (P = 0.596) test, which suggested there was no evidence of publication bias (**Figure 3**).

Discussion

To our knowledge, this is the first meta-analysis evaluating the association between fish intake and HCC risk. The present meta-analysis included 12 observational studies currently available (three cohort studies and nine case-control studies), involving a total of 1,071,458 participants and 2,627 HCC cases. There was statistically significant heterogeneity among the 12 included studies investigating the association between fish consumption and HCC risk, so a random-effects model was chosen over a fixed-effects model. Finally, we found that there was no association between fish consumption and a significant reduction in HCC incidence (comparing the highest with the lowest category). Sensitivity analysis indicated that an omission of any studies did not alter the magnitude of observed effect, suggesting a stability of our findings. Moreover, the results of Begg's test and Egger's test did not support the existence of major publication bias.

As we know, fish oil is rich source of n-3 fatty acids. Previous animal model studies have shown that n-3 polyunsaturated fatty acids were linked to the reduction of the progression of cancer cells [43, 44]. Multiple mechanisms are involved in this chemopreventive activity, including suppression of neoplastic transformation, cell growth inhibition and enhanced apoptosis, and antiangiogenicity [45-47]. Although inverse association between fish intake and risk of HCC is biologically plausible, our result showed a null association. In the present meta-analysis, most of the studies

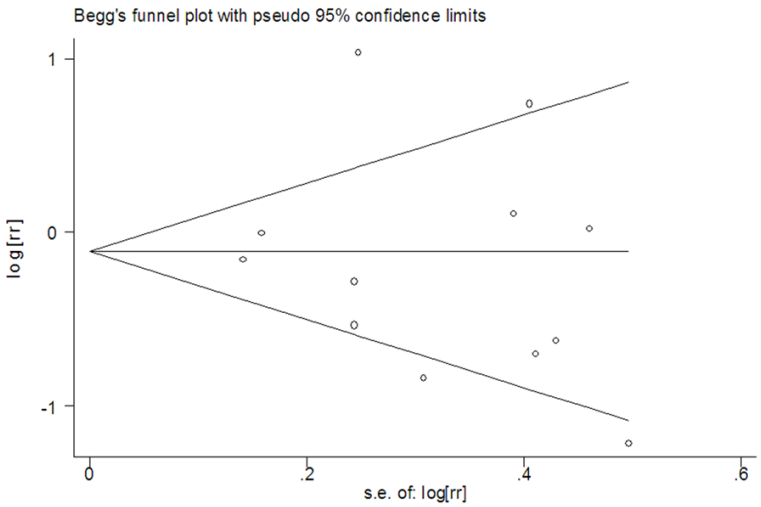

Figure 3. Funnel plot for publication bias in the studies investigating risk for HCC associated with fish intake.

were case-control studies. Our findings from the case-control studies suggested a nonsignificant reduction between fish intake and HCC risk, but the results from the prospective cohort studies showed a statistically significant inverse association. Compared with retrospective case-control studies, prospective cohort studies are less susceptible to bias (e.g. recall bias, selection bias) due to their nature. Furthermore, case-control studies had a lower median quality score than cohort studies (7 versus 9). So, the association may be changed by poor study methodologies. In addition, we should notice that there were only three cohort studies investigating the association between fish intake and the risk of HCC. That number is rather low to draw firm conclusions. So more prospective cohort studies are needed to confirm the association between fish consumption and risk of HCC in the future.

Most of the studies reporting the associations of fish with risk of HCC were primarily designed to study either the effect of meat or a variety of risk factors. Thus, they focused on total fish rather than different species of fish or different preparation methods. This limitation might contribute to the null findings in the primary studies and this meta-analysis. Fish can be served in many ways, such as fresh, broiled, baked, salted, or fried. Fish preparation methods may alter the relation between fish intake and HCC by changing the lipid profile and by generating unexpected chemicals with the use of certain cooking methods. Frying, in particular, was found to considerably reduce the amount of long-chain (n-3) PUFA (LC-PUFA) in fish [48]. Deep-frying could generate trans-fatty acids, oxidized lipids, or food mutagens such as heterocyclic amines and benzo (a) pyrene, which may promote carcinogenesis and which was found to be associated with elevated cancer risk [49]. Salted fish are rich in chemical carcinogens, such as nitrites, heterocyclic amines, 2-chloro-4-methylthiobutanoic acid, and so on, which may be associated with increased risk of cancer [50, 51]. There were four studies [31, 35, 36, 39] investigating processed fish and HCC risk, however, the results of meta-analysis haven't shown a significant association between processed fish intake and increased HCC risk. We think there are several reasons which may lead to the null association. Firstly, all the four studies were retrospective case-control studies. Secondly, three of the four studies have adjusted few confounders ($n \leq 4$). Thirdly, we should notice that there were only four studies investigating the association between processed fish intake and HCC risk, that number is rather low to draw firm conclusion. So more prospective cohort studies with well controlled confounding factors are needed to confirm the association between processed fish consumption and risk of HCC.

The strength of the present meta-analysis lies in a large sample size (2,627 HCC cases and 1,071,458 participants) and no significant evidence of publication bias. Two investigators independently performed the article identification, data extraction,

and verification and resolved all discrepancies. Furthermore, our findings were stable and robust in sensitivity analyses. However, several limitations to this meta-analysis should be noted. Firstly, as a meta-analysis of observational data, the possibility of recall and selection biases cannot be ruled out. Compared with case-control studies, cohort studies are less susceptible to bias due to their nature. However,

the present meta-analysis included only three cohort studies, so more prospective cohort studies are need to confirm the association in the future. Secondly, we did not search for unpublished studies, so only published studies were included in our meta-analysis. Therefore, publication bias may have occurred although no publication bias was indicated from both visualization of the funnel plot and Egger's test. Thirdly, most of the included studies haven't adjusted for hepatitis B and C virus infection, aflatoxin exposure, tobacco smoking, and alcohol abuse accompanied by liver cirrhosis, which are associated with increased risk of HCC. Fourthly, all of the included studies didn't reported results separately for males and females, and we can't do subgroup analysis according to gender. So, future studies should reported results separately for males and females. Lastly, different types of fish (lean fish and fatty fish, fresh fish and processed fish) may have different effects on the risk of HCC, however, we can't do detailed meta-analysis for a lack of data. Although we assessed processed fish and the risk of HCC, the number of included studies is rather low to draw firm conclusion.

In conclusion, the present meta-analysis suggests that there was no association between fish consumption and a significant reduction in HCC incidence. More in-depth studies are warranted to report more detailed results, including stratified results by fish type, preparation methods, and gender.

Disclosure of conflict of interest

None.

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Table S1. The quality scores of cohort studies and case-control studies

Study and year	Selection			Comparability			Exposure			Data analysis that used an energy-adjusted residual or nutrient-density model	Total quality scores
	Adequate definition of cases	Representativeness of cases	Selection of control subjects	Definition of control subjects	Study controls for age/gender	Study controls for additional factors	Exposure assessment	Same method of ascertainment for cases and controls	Non-Response rate		
Kanazir M 2010	☆	—	—	☆	☆	—	☆	☆	☆	—	6
Talamini R 2006	☆	☆	—	☆	☆	☆	☆	☆	☆	☆	9
Yu S 2002	—	—	☆	☆	☆	☆	—	☆	☆	—	6
Fernandez E 1999	☆	☆	—	☆	☆	☆	☆	☆	☆	—	8
Wang ZJ 1996	—	—	☆	☆	☆	☆	☆	☆	☆	—	7
Srivatanakul P 1991	☆	—	☆	—	—	—	☆	☆	☆	—	5
Lu SN 1988	—	☆	☆	☆	☆	☆	☆	☆	☆	—	8
La Vecchia C 1988	☆	☆	☆	☆	—	—	☆	☆	☆	—	7
Lam KC 1982	☆	—	☆	☆	—	—	☆	☆	☆	—	6

Study and year	Selection			Comparability			Outcome			Data analysis that used an energy-adjusted residual or nutrient-density model	Total quality scores
	Representativeness of the exposed cohort	Selection of the unexposed cohort	Ascertainment of exposure	Demonstration that outcome of interest was not present at start of study	Study controls for age/gender	Study controls for additional factors	Assessment of outcome	Was follow-up long enough for outcomes to occur	Adequacy of follow up of cohorts		
Fedirko V 2013	☆	☆	☆	☆		☆	☆	☆	☆		8
Sawada N 2012	☆	☆	☆	☆	☆	☆	☆	☆	☆		9
Daniel CR 2011	☆	☆	☆	☆	☆	☆	☆	☆	☆	☆	10

Methodologic quality of case-control studies included in the meta-analysis.