Published in final edited form as:
Eur J Cancer. 2012 September ; 48(14): 2125–2136. doi:10.1016/j.ejca.2012.02.009.

Population Attributable Risk of Aflatoxin-Related Liver Cancer: Systematic Review and Meta-Analysis

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Abstract

Background—Over 4 billion people worldwide are exposed to dietary aflatoxins, which cause liver cancer (hepatocellular carcinoma, HCC) in humans. However, the population attributable risk (PAR) of aflatoxin-related HCC remains unclear.

Methods—In our systematic review and meta-analysis of epidemiological studies, summary odds ratios (ORs) of aflatoxin-related HCC with 95% confidence intervals were calculated in HBV+ and HBV− individuals, as well as the general population. We calculated the PAR of aflatoxin-related HCC for each study as well as the combined studies, accounting for HBV status.

Results—17 studies with 1680 HCC cases and 3052 controls were identified from 479 articles. All eligible studies were conducted in China, Taiwan, or sub-Saharan Africa. The PAR of aflatoxin-related HCC was estimated at 17% (14–19%) overall, and higher in HBV+ (21%) than HBV− (8.8%) populations. If the one study that contributed most to heterogeneity in the analysis is excluded, the summarized OR of HCC with 95% CI is 73.0 (36.0–148.3) from the combined effects of aflatoxin and HBV, 11.3 (6.75–18.9) from HBV only, and 6.37 (3.74–10.86) from aflatoxin only. The PAR of aflatoxin-related HCC increases to 23% (21–24%). The PAR has decreased over time in certain Taiwanese and Chinese populations.

Conclusions—In high exposure areas, aflatoxin multiplicatively interacts with HBV to induce HCC; reducing aflatoxin exposure to non-detectable levels could reduce HCC cases in high-risk areas by about 23%. The decreasing PAR of aflatoxin-related HCC reflects the benefits of public health interventions to reduce aflatoxin and HBV.

Keywords

Aflatoxin; hepatocellular carcinoma; hepatitis B virus; population attributable risk; systematic review; meta-analysis

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Conflicts of Interests

The authors declare that they have no competing financial interests.

INTRODUCTION

Aflatoxins are toxic and carcinogenic chemicals produced primarily by the fungi *Aspergillus flavus* and *A. parasiticus,* which infect food crops such as maize, peanuts, and tree nuts. About 4.5 billion people worldwide are exposed to dietary aflatoxins (1). Exposures are highest in tropical and subtropical regions of the world, where maize and peanuts are dietary staples and food storage conditions are suboptimal (1, 2).

Aflatoxins are among the most potent naturally occurring human hepatocarcinogens known. The International Agency for Research on Cancer (IARC) has classified "naturally occurring mixes of aflatoxins" as a Group 1 human carcinogen (3). Abundant epidemiological evidence suggests that aflatoxin exposure synergizes with chronic hepatitis B virus (HBV) infection to increase liver cancer (hepatocellular carcinoma, HCC) risk in populations with both risk factors (4–8). More recently, toxicological models for the mechanism of the synergism of these two risk factors have emerged (9–11), and are summarized in Wild and Gong (12). Unfortunately, both high aflatoxin exposure and HBV are prevalent in many parts of the developing world, particularly Asia and Africa.

Previously, by compiling food consumption and aflatoxin contamination data in multiple countries and conducting a quantitative cancer risk assessment, we estimated that 25,200–155,000 (5–28%) annual HCC cases worldwide could be attributed to aflatoxin exposure (13). This large range highlights the limitations in obtaining exposures solely from food surveys, uncertainties in the nature of the dose-response relationship, and uncertainties in HBV prevalence data in different nations.

In this context, systematically analyzing human studies that relate biomarkers of aflatoxin exposure and HBV infection to HCC may provide a more precise and accurate measurement of burden of HCC caused by aflatoxin. Therefore, in this study, we systematically reviewed epidemiological studies on these associations in different world regions. By combining the relevant odds ratios (ORs) and relative risks (RRs) from these studies, we conducted meta-analyses to calculate population-attributable risk (PAR) of aflatoxin-related HCC in the population overall, as well as in HBV+ and HBV− populations. PAR is the proportion of disease cases that could be avoided if a particular risk factor were eliminated in a population. In the context of our study, PAR of aflatoxin-related HCC is the proportion of HCC cases that could be avoided in a chosen population by reducing aflatoxin exposures (as measured by biomarkers) from detectable to undetectable levels.

METHODS

Search Strategy

We performed a literature search until May 13th, 2011, using the following search terms on Medline/PubMed: (aflatoxin) and (hepatitis B) and (liver cancer); (aflatoxin) and (hepatitis B) and (hepatocellular carcinoma). Additionally, we searched reference lists from retrieved articles to identify further relevant studies. Our systematic review and meta-analyses were conducted in adherence to PRISMA standards for reporting meta-analyses (14).

Eligibility Criteria

Studies were included in the systematic review if they met the following criteria: (1) case-control or cohort study design; (2) aflatoxin as the exposure of interest; (3) HBV as the infection of interest (hepatitis B virus surface antigen [HBsAg] as a marker of chronic HBV infection); (4) HCC as the outcome of interest; and (5) relative risk (RR) or odds ratio (OR) estimates with 95% confidence intervals (CIs) reported, or data to calculate these.

Data Extraction

The following data were extracted from each study: authors, publication year, study design and sample size, study location, study period, participants' gender and age range, metric and range of aflatoxin exposure, estimated adjusted RRs/ORs, and variables adjusted for analysis. Because all identified studies are case-control designs except one cohort study, and because RR and OR can be used interchangeably when the disease is relatively rare (<15%; HCC rates are lower than this in the populations studied), we combined the RR from this study with the ORs from the case-control studies to calculate a summary OR. If aflatoxin exposure was measured using different biomarkers in the same study, we selected the ones reflecting consistent biomarkers amongst different studies (one OR per study was used).

Statistical Methods for Meta-analysis

The ORs from the studies were first combined in a meta-analysis using a random-effects model, and then a fixed-effects model if heterogeneity in the study pool was insignificant (15). The studies were categorized by the recruited population type: general populations, and HBV+ or HBV− populations. First, all the studies providing data for general populations (including both HBV+ and HBV− individuals) were combined, and ORs of aflatoxin-related HCC after HBsAg+ adjustment and ORs for combined (aflatoxin+HBV) effects were analyzed. Then the studies with data from HBV+ populations (and studies that recruited from the general population but separately estimated ORs in HBV+ populations) were combined; and the ORs for HBV+ populations only were estimated. We also combined the studies that separately estimated the ORs in HBV− populations. If the study examined the association between aflatoxin exposure and HCC in various exposure categories, we chose the ORs reflecting highest and lowest levels of aflatoxin exposure for the meta-analysis.

Heterogeneity amongst the studies was evaluated using the Cochran's Q value calculated from the Mantel-Haenszel method and the I^2 statistic (15). We performed sensitivity analyses in which each study was in turn removed and the rest analyzed to evaluate if the results were significantly affected by one particular study. Publication bias was assessed by a funnel plot and associated statistical tests of asymmetry. All statistical analyses were performed with Comprehensive Meta-Analysis software Version 2.2.

Statistical Methods for PAR Calculations

We estimated the PAR for aflatoxin-related HCC in HBV+ and HBV− populations for each study if the data were available. To estimate the PAR for aflatoxin-related HCC using the adjusted ORs, we used the attributable fraction formula (16):

$$AF_{pop}=\sum_{i=1}^{z} W_i \frac{P_c(RR_i-1)}{1+P_i(RR_i-1)}$$

where AF_{pop} is aflatoxin attributable risk fraction in the population including exposed and unexposed individuals, P_i is the proportion of the population in stratum i that is exposed, and W_i is the proportion of diseased individuals (cases) in stratum i. We use adjusted OR_i in stratum i as an approximation of RR_i.

If the study provided risk estimates adjusted by HBsAg positivity, we used the formula below (16) to estimate the PAR of aflatoxin-related HCC in the general population:

$$AF_{pop}=\frac{P_c(RR-1)}{RR}$$

where P_C is the proportion of cases exposed in the combined population based on detection limits for aflatoxin biomarkers in the studies, and HBsAg positivity-adjusted OR is used as an approximation of RR. For each AF_{pop}, we calculated 95% confidence intervals (CI) using the method described in Daly (17).

RESULTS

Literature Search

The step-by-step process of our literature search is presented in Figure 1. From 479 results, we excluded human cell line studies, animal studies, and review articles. Using the eligibility criteria described above, 27 studies were selected. Three more relevant studies were identified from the reference lists of the 27 selected studies. We then read the full texts of these 30 studies. Six studies were excluded because they were duplicated reports from the same population in the same time period, and seven more were excluded because quantitative measurements of association between aflatoxin exposure and HCC were not provided. Thus, 17 studies were included in this systematic review and PAR analysis.

Study Characteristics

Table 1 provides an overview of the eligible studies. The 17 studies (5–8,18–30) on aflatoxin exposure and HCC risk - eight case-control studies, eight nested case-control studies, and one cohort study - were published between 1994 and 2009. There were 1680 HCC cases and 3052 controls in total.

Four studies reported results for one Taiwanese cohort from four different time periods (7,18,25,30) from 1980s to 2000s. To determine if all these studies should be included in the meta-analysis, we first examined the heterogeneity between the risk estimates provided by these studies. Because of the significant heterogeneity of aflatoxin exposures and HCC risk estimates in this cohort between the follow-up studies through the years, we treated these as independent studies in the analysis. In analyses that included only the most recent of all studies in a particular cohort, the results were nearly identical to those obtained when including all studies (Table 2). Two articles reported results from one case-control study in Sudan from different perspectives (risk estimates for the general population after adjustment of HBsAg+, and risk estimates for HBsAg+ or HBsAg− separately) (8,24). Likewise, two articles reported results from a study in The Gambia with risk estimates for the general population after adjustment of HBsAg+, and risk estimates for HBsAg+ or HBsAg− separately (5,23).

Aflatoxin Exposure and HCC Risk by HBsAg Status

The association between aflatoxin exposure and HCC, independently or in conjunction with HBV, was analyzed by combining eligible studies by HBsAg+ status and calculating summary ORs (Table 2). Meta-analyses were conducted by geographic region (China, Taiwan, and sub-Saharan Africa).

Aflatoxin exposure is significantly associated with HCC risk, regardless of HBsAg status, with a summarized OR of 4.75 (2.78–8.11) from nine studies in the general population adjusted by HBsAg positivity, 2.39 (1.50–3.82) from eleven studies in HBsAg+ populations and 5.91 (3.66–9.55) from six studies in HBsAg− populations.

Sensitivity Analysis

For the meta-analysis of aflatoxin-related HCC risk in the general population, our sensitivity analyses revealed that Wu et al. (30) was the most influential study in determining the

summarized OR. After excluding this particular study, heterogeneity was significantly reduced (Q=8.40, P=0.30, I^2=16.66), and the summarized OR was 5.57 (3.78–7.79).

For the meta-analysis of aflatoxin exposure and HCC in HBsAg+ populations, our sensitivity analyses showed that two studies, Szymanska et al. (29) and Wu et al. (30), substantially influenced the summarized OR. After excluding the two studies, heterogeneity was significantly reduced (Q=11.16, P=0.19, I^2=28.29), and the summarized OR of HCC risk for detectable vs. non-detectable aflatoxin exposure in HBsAg+ individuals was 2.90 (2.09–4.01). These results suggest that the two studies that measured the association between HCC and aflatoxin exposure in the most recent years (29,30) appear to have significantly different results from relatively earlier studies.

For the 10 studies (6,7,18,20,22–24,27,28,30) associating aflatoxin and liver cancer in the general population, we assessed publication or other forms of selection bias by a funnel plot (Figure 3) and associated statistical tests of funnel plot asymmetry (31). Seven studies are not included in this plot; five studied the association in HBsAg+ individuals only, and two are duplicate studies included in meta-analysis for different data extraction purposes, as explained in the Methods. The funnel plot provides little evidence of an important departure from symmetry, indicating that publication or other forms of selection bias were not a serious limitation in our meta-analysis. This visual impression of symmetry was corroborated by the statistical tests of funnel plot asymmetry.

Multiplicative Model of Effects between Aflatoxin Exposure and Chronic HBV Infection

The meta-analysis allowed us to quantitatively evaluate the model of effects between the two risk factors aflatoxin and HBV in liver cancer. The summary OR of six studies (5–8,20,30) reporting ORs of HCC risk from both aflatoxin exposure and HBV is 54.1 (21.3–137.7) with significant heterogeneity (Q=13.65, P=0.02, I^2=63.36). The summary OR of the same group of studies for HCC from aflatoxin exposure alone is 5.91 (3.66–9.55), while the summary OR on HCC risk from chronic HBV alone is 11.2 (7.48–16.7), both with no significant heterogeneity. When we excluded Wu et al (30) which contributes most to the heterogeneity, the summarized OR for combined effects increased to 73.0 (36.0–148.3), 6.37 (3.74–10.86) for aflatoxin exposure alone, and 11.3 (6.75–18.9) for chronic HBV infection alone (Figure 3). These estimates indicate an almost perfectly multiplicative model of effects between aflatoxin exposure and chronic HBV in HCC risk.

PAR of HCC from Aflatoxin Exposure in Each Study Population

The PAR of aflatoxin-related HCC was calculated for each study population (Table 3). PAR is the proportion of the HCC cases that could be prevented by reducing aflatoxin exposures to “control” levels in each study. For example, HCC in the Chen et al (18) Taiwanese study population could be reduced by about 10% (2.5–12%) if dietary aflatoxin exposures in this population were reduced such that aflatoxin-albumin adduct levels were below 0.01 fmol/μg (detection limit in this study), or if dietary aflatoxin exposures could be decreased to below 4.3 ng/kg bw/day (biomarker detection limit extrapolated to dietary exposure). HCC in the study population of Shanghai males in Qian et al. (6) could be reduced by about 9.0% (5.9–10.4%) if aflatoxin exposures in this population were reduced to below 6 ng/kg bw/day: the average aflatoxin exposure level in the control group. Our results showed that the PAR of HCC caused by aflatoxin is higher in HBV+ populations than in HBV− populations.

In HBV+ populations in a Taiwanese cohort, the PAR for aflatoxin-related HCC is consistently decreasing, as indicated by a series of follow-up studies: 31% in 1980s (7), 12% in 1990s (25), and 3% in 2000s (30). Overall, the PAR of aflatoxin-related HCC is

decreasing in Taiwan in both HBV+ and HBV− individuals, from as high as 44% in 1990s (20) to 2% in 2000s (30).

We combined all aflatoxin-exposed cases, HBV+ and HBV− individuals, and controls from all eligible studies to calculate the PAR of aflatoxin-related HCC by HBsAg status and world region (Tables 4,5). The PAR of aflatoxin-related HCC in the general population after HBV adjustment is 17% (14–19%). Because the earlier sensitivity analysis demonstrated that the remaining studies after exclusion of Wu et al. (30) do not have statistically significant heterogeneity, we also calculated the PAR of aflatoxin-related HCC after exclusion of (30). The PAR increased to 23% (21–24%).

The PAR of aflatoxin-related HCC in the HBV+ population is 21% (10–29%). A separate calculation was performed excluding Szymanska et al (29) and Wu et al (30), the most influential studies indicated by the sensitivity analysis. The new PAR of aflatoxin-related HCC in the HBV+ population was 25% (18–30%). The PAR of aflatoxin-related HCC in HBV− populations is 8.8% (6.7–10%).

Discussion

Aflatoxin exposure is significantly associated with HCC risk regardless of HBV status. Our meta-analyses show that in areas of high aflatoxin exposure and chronic HBV infection, aflatoxin exposure and HBV have a nearly perfectly multiplicative relationship in increasing HCC risk. In populations including both HBV+ and HBV− individuals in the geographic regions studied, the PAR of aflatoxin-related HCC was estimated at 17% (14–19%). This implies that if it were possible to reduce aflatoxin to below detectable limits in these regions, HCC incidence could be reduced by 14–19%. There are roughly 520,000 new HCC cases in China, southeastern Asia and sub-Saharan Africa each year (32). If the PARs are generalized to these areas, the implication is that, by reducing aflatoxin in human diets to below detectable levels, 72,800 to 98,800 new HCC cases could be prevented every year. If this PAR were generalized to regions of the world beyond Africa and Asia, the overall number of HCC cases (749,000 new cases per year (32)) that could be prevented by aflatoxin control would reach 105,000–142,000.

The PAR of aflatoxin-related HCC increases to 23% (21–24%), and heterogeneity amongst the studies decreases significantly, if one study (30) is excluded from the meta-analysis. However, this study is important because it suggests that aflatoxin exposure is decreasing over time in the Taiwanese (Penghu) population studied. Our PAR estimates for individual studies showed a decrease in PAR of aflatoxin-related HCC in the Penghu cohort in the last three decades. It is worth noting that in a 1970s food survey, over one-third of peanuts in Penghu were heavily contaminated by aflatoxins, with an average aflatoxin content of 167 μg/kg (33). Mean urinary aflatoxin in HCC patients in this cohort from was 219 μg/ml in 1991/1992 (7,18), and decreased to 0.017 μg/ml in HCC patients in the same cohort in 2004 (30). Also, the HBV vaccination program in Taiwan has successfully reduced HBV prevalence, further reducing HCC risk (34).

In some parts of the world such as Taiwan, aflatoxin exposure is decreasing. In other parts of the world such as Africa, rural China, and Southeast Asia, there is little evidence that aflatoxin exposure is decreasing; in fact, two recent Kenyan events of extremely high aflatoxin levels in maize (in 2004–2005, and again in 2010) suggest the opposite. With climate change, aflatoxin contamination in food crops may become exacerbated due to conditions favoring proliferation of Aspergilli (35). Hence, further efforts to reduce aflatoxin-related disease are needed in high-risk areas of the world.

There are several limitations in this analysis. First, the epidemiological studies included were conducted in areas of the world with both high aflatoxin and HBV (Asia and sub-Saharan Africa). Thus, although these regions account for most of the aflatoxin-induced HCC cases worldwide (13), the estimated PAR is not necessarily applicable in areas with much lower aflatoxin exposures. Second, odds ratios from studies employing food surveys, exposure biomarkers and biological effect biomarkers were combined. This decreases the precision of the analysis, as different biomarkers have different detection limits and measure different endpoints, and food surveys are less precise than biomarkers for exposure estimation. Third, the PAR is meant to represent the proportion by which disease could be reduced if the risk factor in question were removed. It is not possible to instantaneously reduce aflatoxin to below detectable limits worldwide – rather, the PAR calculated is meant to estimate the burden of HCC caused by one risk factor (aflatoxin), and to project the extent to which the problem could be reduced in future generations if aflatoxin control strategies were widespread.

In summary, this study is the first to quantitatively evaluate the model of effects between aflatoxin and HBV in inducing liver cancer by combining results from multiple epidemiological studies. The range of PARs calculated in this analysis, 14–19% (21–24% excluding one study contributing to heterogeneity), is consistent with our previous report of 5–28% using a different methodology (quantitative cancer risk assessment) (13). The PAR of aflatoxin-related HCC is higher in HBsAg+ populations than HBsAg− populations. In recent years, the PAR of aflatoxin-related HCC has shown a decreasing trend in areas such as Taiwan, indicating the benefits of reduced aflatoxin exposure and HBV prevalence by public health interventions.

Acknowledgments

Funding: US National Cancer Institute (5R01CA153073-02), National Institutes of Health

Abbreviations

AFB_1	Aflatoxin B_1
PAR	Population attributable risk
OR	Odds ratio
RR	Relative risk
HBV	Hepatitis B virus
HBsAg	Hepatitis B virus surface antigen
HCC	Hepatocellular carcinoma
HCV	Hepatitis C virus
AFM_1	Aflatoxin M_1
IARC	International Agency for Research on Cancer
JECFA	Joint Food and Agriculture Organization/World Health Organization Expert Committee on Food Additives

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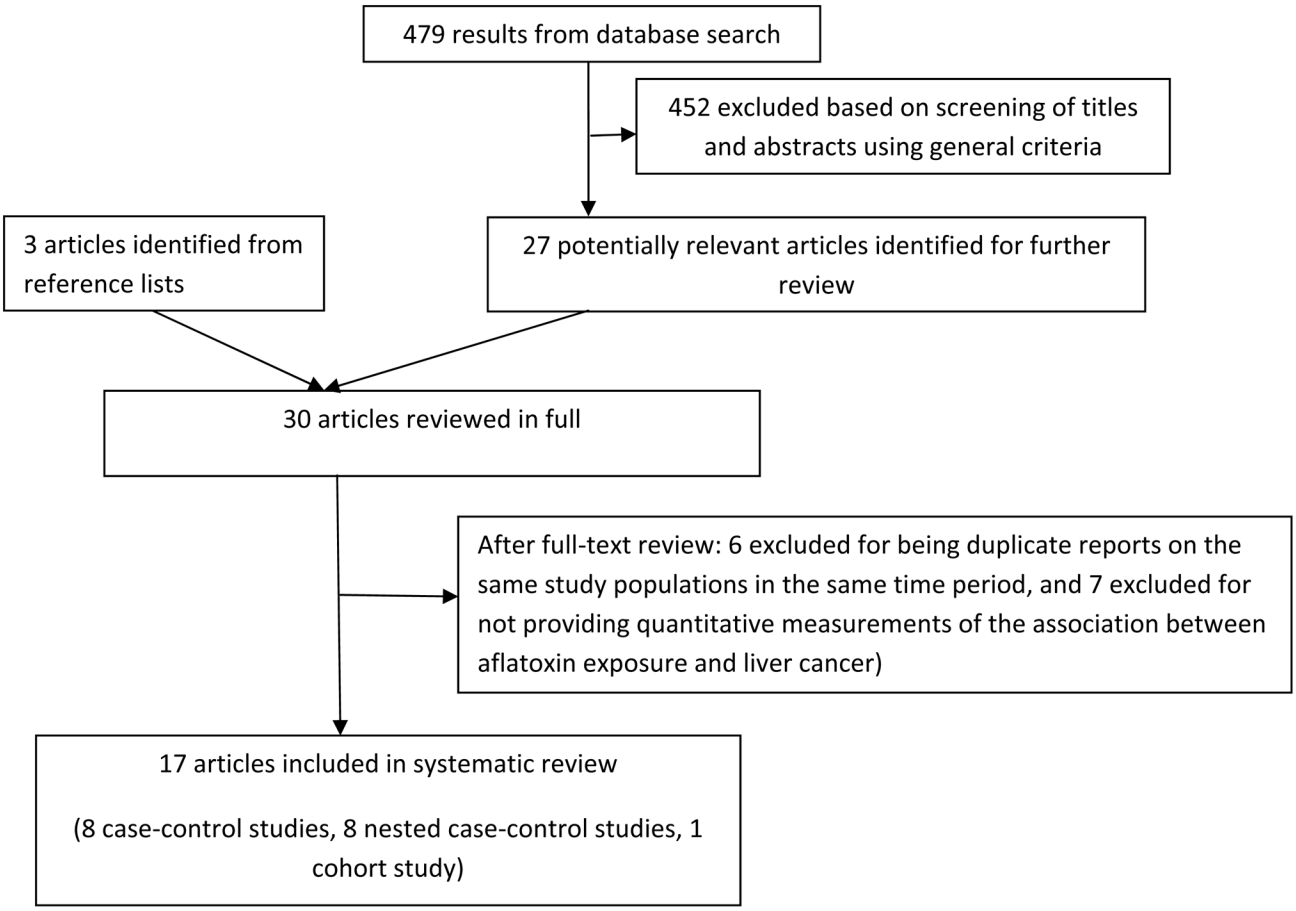

Figure 1.
Selection of studies for inclusion in systematic review

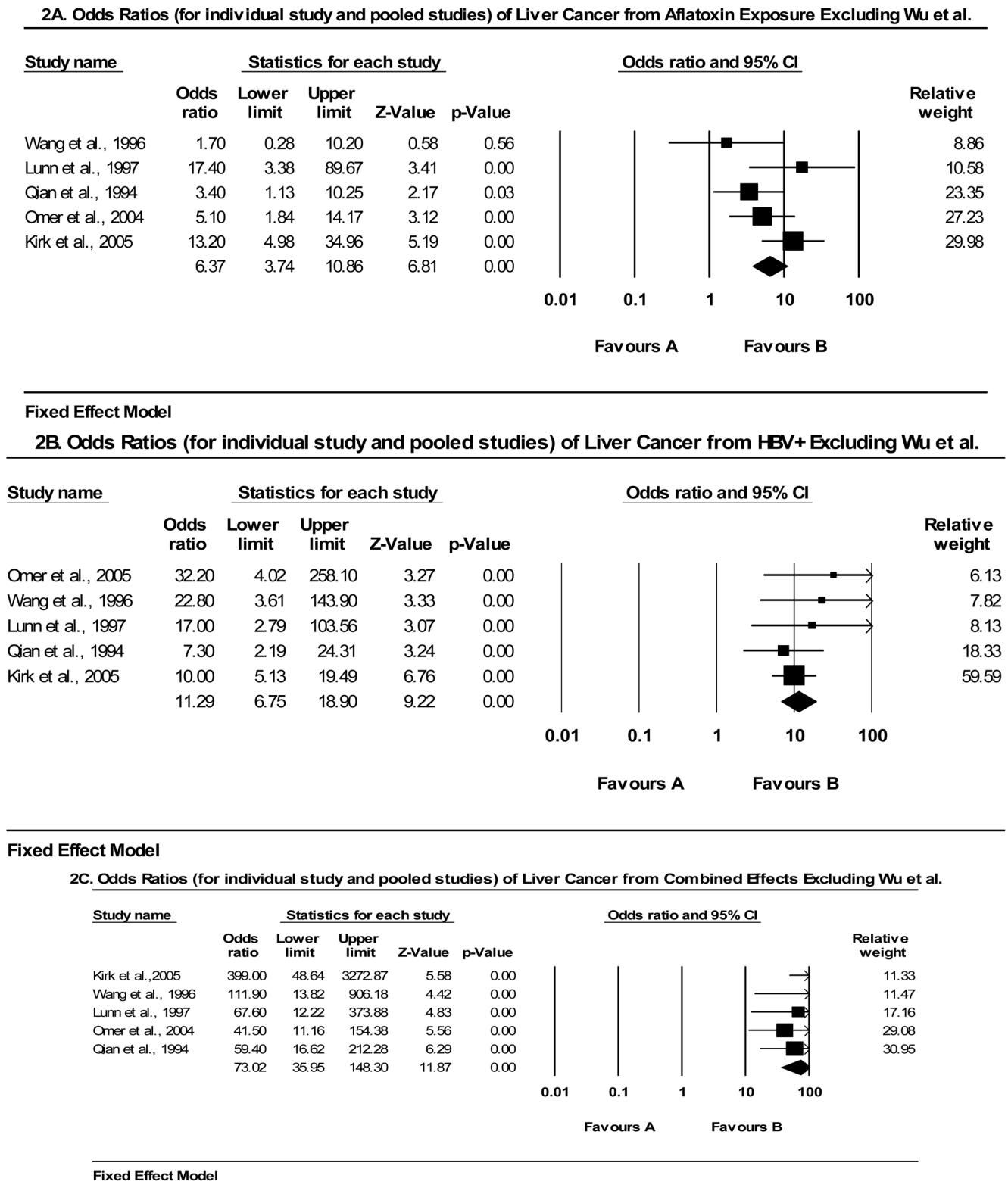

Figure 2. Odds ratios (ORs) and 95% CIs for association between liver cancer and two risk factors (aflatoxin exposure and chronic HBV), independently and in combination

Squares and horizontal lines correspond to the study-specific OR and 95% CI; the box size is proportional to the meta-analysis study weight; diamonds represent summarized ORs. 2A: ORs with 95% CI for association between liver cancer and chronic HBV+ only, excluding Wu et al (30).2 B: ORs with 95% CI for association between liver cancer and aflatoxin exposure only, excluding Wu et al (30). 2C: ORs with 95% CI for association between liver cancer and the combination effects of two risk factors, excluding Wu et al (30).

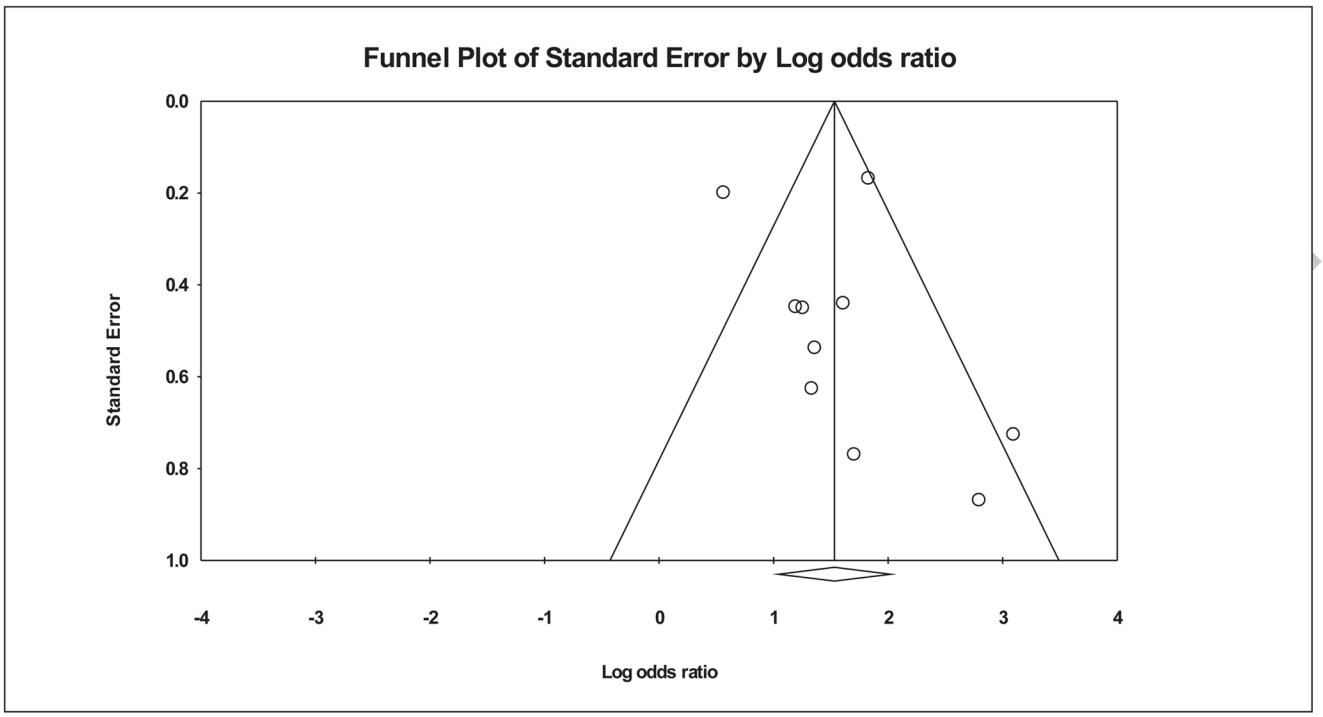

Figure 3. Funnel plot to assess possible publication or other selection bias for the association between aflatoxin exposure and liver cancer risk in general population

No statistically significant asymmetry was found. Each circle represents 1 study. 10 studies (6, 7, 18, 20, 22–24, 27, 28, 30) are eligible for this plot. 7 studies not included (5 only studied the association in HBsAg+ individuals, and 2 are duplicate studies included in meta-analysis for different data extraction purpose, as explained in the Methods section).

Table 1

Characteristics of the eligible studies included in the systematic review/meta-analysis[a,b]

No	Source	Location/period	Sex	Age, yrs	No of Cases (% exposed)	No of Controls (% exposed)	Measure/Range of Exposure, detection limit	Adjusted ORs/RRs[c]	Adjustment for Covariates
1	Qian et al., 1994 (6) (cohort of 18,244 middle-aged men)	China, 1986–1992	M	45–64	50 cases (36%)	267 matched controls (12%)	AFB_1-N^7-Gua adduct (detectable vs non-detectable, 0.07 ng aflatoxins/ml urine	9.1 (2.9–29.2)	HBsAg positivity, cigarette smoking
					50 cases (72%)	267 matched controls (41%)	Multiple urinary biomarker (detectable vs non-detectable, 0.01 fmol/μg)	5.0 (2.1–11.8)	
2	Chen et al., 1996 (18) (7 township cohort nested case-control study)	Taiwan, 1991–1992	F/M	36–65	20 cases (65%)	86 matched controls (37%)	AFB_1-albumin adducts (detectable Vs non-detectable, 0.01 fmol/ μg)	5.5 (1.2–24.5)	HBsAg, anti-HCV, family history of liver cancer cirrhosis
3	Chen et al., 1996 (19) (nested case-control in cohort of 4,841 male HBsAg individuals)	Taiwan, 1988–1992	M	30–65	32 cases (37.5% low exposure)	73 matched controls (33% low exposure)	AFB_1-albumin adducts (Low Vs Non-detectable, 0.01 fmol/ μg)	1.6 (0.6–4.0)	Cigarette smoking, alcohol consumption
					32 cases (19% high exposure)	73 matched controls (6.8% high exposure)	AFB_1-albumin adducts (High Vs Non-detectable, 0.01 fmol/ μg)	3.8 (1.0–14.5)	
4	Wang et al., 1996 (7) (7 township cohort nested case-control study	Taiwan, 1991–1995	F/M	30–64	52 cases (60%)	168 matched controls (37%)	AFB_1-albumin adducts (detectable vs non-detectable, 0.01 fmol/ μg)	1.6 (0.4–5.5)	HBsAg positivity
					38 cases (53%)	137 matched controls (45%)	Urinary aflatoxin metabolite (high vs low, 0.01 fmol/μg)	3.8 (1.1–12.8)	
5	Zhang et al., 1997 (22) (Hospital-based case-control study)	China, 1994–1995	F/M	18–88	152 cases (33%)	115 non-hepatic patient controls (2%)	Corn consumption history from dietary questionnaire	(1:1 pair-matched) 16.44 (1.67–61.65)	HBV infection, individual history of liver diseases, family history of liver diseases, and peanut consumption
					152 cases (89%)	115 non-hepatic patient controls	Peanut consumption history from dietary questionnaire (49%)	3.51 (1.45–8.47)	HBV infection, individual history of liver diseases, family history of liver diseases, and corn consumption

No	Source	Location/period	Sex	Age, yrs	No of Cases (% exposed)	No of Controls (% exposed)	Measure/Range of Exposure, detection limit	Adjusted ORs/RRs[c]	Adjustment for Covariates
6	Yu et al., 1997 (21) (nested case-control of a cohort of 4841 male HBsAg individuals)	Taiwan, 1988–1994	M	30–65	42 cases (29%)	43 matched controls (14%)	AFB_1-N^7-gua, (below 0.21 ng/ml Vs 0.21–0.36 ng/ml, 0.05 ng aflatoxin/ml urine)	5.3 (1.1–25.2)	Education level, ethnicity, habitual alcohol drinking and cigarette smoking status
					42 cases (14%)	43 matched controls (16%)	AFB_1-N^7-gua (below 0.21 ng/ml Vs >0.36 ng/ml, 0.05 ng aflatoxin/ml urine)	2.8 (0.6–12.9)	
					42 cases (24%)	43 matched controls (23%)	AFM_1 (below 1.61 ng/ml Vs 1.61–2.85 ng/ml, 0.05 ng aflatoxin/ml urine)	1.9 (0.5–7.2)	
					42 cases (55%)	43 matched controls (35%)	AFM_1 (below 1.61 ng/ml Vs >2.85 ng/ml, 0.05 ng aflatoxin/ml urine)	6.0 (1.2–29.0)	
7	Lunn et al., 1997 (20) (case-control study)	Taiwan, 1984–1995	F/M		105 cases (80%)	37 controls (43%)	AFB_1-DNA adducts	Corrected OR: 3.9 (1.4–11.5)	n/a
8	Kirk et al., 2000 (23) (case-control study)	The Gambia, 1997–1998	F/M	20–73	53 cases (36%)	53 matched controls (5.7%)	Ser-249 P53 mutation	16.4 (3.0–90.5)	Age, sex, recruitment site and HBsAg positivity
9	Sun et al., 2001 (25) (7 township cohort nested case-control study, HBsAg individuals)	Taiwan, 1991–1997	F/M	30–64	75 cases (64%)	140 matched controls (46%)	Aflatoxin-albumin adducts (detectable vs non-detectable, 0.01 fmol/μg)	2.0 (1.1–3.7)	Sex, age and residence
10	Omer et al., 2001 (24) (case-control study)	Sudan 1996–1998	F/M	20–70	115 cases	199 matched controls	Peanut butter consumption >300 g/mo Vs Peanut butter consumption <70 g/mo	3.3 (1.4–8.1)	Age and hepatitis
11	Ming et al., 2002 (26) (Hospital-based cohort, 145 HBsAg individuals)	Qidong, China	M	27–74	31 cases	145 HBsAg+ carriers follow up	AFM_1 (>3.6 ng/l)	3.5 (1.5–8.1)	Age, HCV, family history of HCC

No	Source	Location/period	Sex	Age, yrs	No of Cases (% exposed)	No of Controls (% exposed)	Measure/Range of Exposure, detection limit	Adjusted ORs/RRs[c]	Adjustment for Covariates
12	Huang et al., 2003 (27) (case-control study)	Qidong, China	F/M	19–87	25 cases (40%)	30 controls (6.7%)	Ser 249 TP 53 mutation	22.1 (3.2–91.7)	Sex, age, recruitment site and HBsAg positivity
13	Omer et al., 2004 (8) (case-control study)	Sudan, 1996–1998	F/M	20–70	114 cases (46%)	198 matched controls (26%)	Peanut butter consumption >300 g/mo Vs Peanut butter consumption <70 g/mo	n/a	age
14	Kirk et al., 2005 (5) (case-control study)	Gambia,	F/M		186 cases (40%)	348 matched controls (3.4%)	Ser-249 TP53 mutation	20.3 (8.19–50.0)	Adjusted for study group, season of recruitment and daily groundnut intake
15	Long et al., 2009 (28) (hospital-based case-control)	China, 2006–2008	F/M	12.1% <35, 77.8% 35–65, 10.1% >65	618 cases (28%)	712 matched control (29%)	AFB_1-adduct: Low (≤ 1·00 μmol/mol DNA) Vs Medium (1.01–2.00 μmol/mol DNA), (0.25 μmol/mol DNA)	2.11 (1.54–2.90)	Age, sex, ethnicity, HBsAg, anti-HCV, and AFB_1–exposure years
					618 cases (47%)	712 matched controls (17%)	AFB_1-adduct: Low (≤ 1.00 μmol/mol DNA) Vs High (≥ 2.01 μmol/mol DNA) (0.25 μmol/mol DNA)	6.23 (4.48–8.67)	
16	Wu et al., 2009 (30) (7 township cohort nested case-control study)	Taiwan 1991–2004	F/M	30–64 yr	230 cases (93%)	1052 matched controls (95%)	AFB_1-albumin adduct (fmol/mg): Non-detectable Vs Detectable (0·01 fmol/μg or 1 fmol/ml)	0.99 (0.48–2.02)	HBsAg, anti-HCV, habitual smoking, alcohol drinking, BMI and the batch of aflatoxin biomarker assay
					230 cases (33%)	1052 matched controls (33%)	AFB_1-albumin adduct (fmol/mg): Below the mean (<59·8) Vs. Above the mean (≥ 59·8),	1.54 (1.01–2.36)	
					198 cases (88%)	904 matched controls (88%)	Urinary AFB_1 metabolites (fmol/ml): Non-detectable Vs Detectable (0.01 fmol/μg or 1 fmol/ml)	1.70 (0.89–3.25)	
					198 cases (57%)	904 matched controls (44%)	Urinary AFB_1 metabolites: Below the mean Vs Above the mean	1.76 (1.18–2.58)	
17	Szymanska et al., 2009 (29) (nested	China, 1989–1998	M	30–59	126 cases (67%)	123 matched controls (68%)	AF-albumin Detectable Vs non-detectable (3 pg/mg)	0.90 (0.52–1.56)	In HBV individuals

Eur J Cancer. Author manuscript; available in PMC 2013 September 01.

No	Source	Location/period	Sex	Age, yrs	No of Cases (% exposed)	No of Controls (% exposed)	Measure/Range of Exposure, detection limit	Adjusted ORs/RRs[c]	Adjustment for Covariates
	case-control study)								

[a] All the eligible studies were conducted in China (6), Taiwan (7), or sub-Saharan Africa (4). Fourteen studies reported biomarker measurements for aflatoxin exposure, while the other three studies relied on food consumption data. Twelve studies included both HBsAg+ and HBsAg− individuals, with risk estimates that were adjusted for HBsAg positivity (nine studies). Five studies were conducted in HBsAg+ populations only.

[b] Among the fourteen studies that utilized biomarkers, five measured urinary aflatoxin biomarkers, including AFM_1 and AFB_1-N^7-Guanine, six measured AFB_1-albumin adducts, two measured AFB_1-DNA adducts, and three measured TP53 249^{ser} mutations. Several studies included measures of more than one biomarker.

[c] 15 out of 16 identified case-control studies provided matched ORs.

Table 2

Summary of combined odds ratios in the meta-analysis

Risk factor	Study Population	Study area (n of studies)	Cases/controls[d]	Odds Ratio, 95% CI	Model	Heterogeneity
Aflatoxin only	General population with HBsAg+ adjustment	China (4) (6, 22, 27, 28)	634 cases/913 controls	5.99 (3.70–9.69)	Fixed	Q=4.86, P=0.18, I^2=38.32
		Taiwan (3)[e] (7, 18, 30)	198 cases/904 controls	2.01 (1.40–2.89)	Fixed	Q=3.19, P=0.20, I^2=37.29
		Sub-Saharan Africa (2) (23, 24)	168 cases/252 controls	4.62 (2.12–10.08)	Fixed	Q=2·69, P=0·1, I^2=62·82
		Summary (9)	1000 cases/2069 controls	4.75 (2.78–8.11)	Random	Q=32.73, P<0·000, I^2=75.56
	General population with HBsAg+ adjustment after adjust heterogeneity	Summary (8) (5–8, 18, 22, 27, 28)	840 cases/1302 controls	5.72 (4.42–7.40)	Fixed	Q=8.40, P=0.30, I^2=16.66
	General population with HBsAg+ adjustment by only including Wu et al as follow-up for cohort in Taiwan	Summary (7) (5, 6, 8, 22, 27, 28, 30)	1000 cases/2069 controls	4.88 (2.62–9.10)	Random	Q=32.55, P<0.000, I^2=81.57
	General population with HBsAg+ adjustment by taking the average effect of the series of Taiwan Studies[f]	Summary (7)	1000 cases/2069 controls	4.92 (2.74–8.82)	Random	Q=29.48, P<0.000, I^2=79.65
	HBsAg+ individuals	China (3) (6, 26, 29)	189 cases/268 controls	2.00 (0.84–4.75)	Random	Q=10·66, P=0·005, I^2=81·24
		Taiwan (6) (7, 19–21, 25, 30)	254 cases/310 controls	1.81 (1.29–2.56)	Fixed	Q=8.38, P=0.14, I^2=40.35
		Sub-Saharan Africa (2) (5, 8)	128 cases/56 controls	6.48 (0.22–194)	Random	Q=6.54, P=0.01, I^2=84.71
		Summary (11)[g]	571 cases/634 controls	2.39 (1.50–3.82)	Random	Q=27.99, P=0.002, I^2=64.27
	HBsAg+ individuals after adjust heterogeneity	Summary (9) (5–8, 19–21, 25, 26)	377 cases/383 controls	2.90 (2.09–4.01)	Fixed	Q=11.16, P=0.19, I^2=28.29
	HBsAg+ individuals by only including most recent follow-up studies in a cohort of Taiwan	Summary (8) (5, 6, 8, 20, 21, 26, 29, 30)	571 cases/634 controls	2.27 (1.24–4.14)	Random	Q=24.33, P=0.001, I^2=71.23
	HBsAg+ individuals by only combing studies with adjusted ORs	Summary	332 cases/538 controls	2.10 (1.25–3.52)	Random	Q=16.40, P=0.012, I^2=63.42
	HBsAg+ individuals by taking the average effect of all follow-up studies in the same cohort [h]	Summary (8)	571 cases/634 controls	2.35 (1.38–3.99)	Random	Q=23·17, P=0·002, I^2=69.79
	HBsAg− individuals	China (1) (6)	18 cases/236 controls	3·4 (1·13–10·25)	/	/
		Taiwan (3) (7, 20, 30)	81 cases/664 controls	5·00 (2·22–11·28)	Fixed	Q=3·69, P=0·16, I^2=45·79
		Sub-Saharan Africa (2) (5, 8)	122 cases/391 controls	8·40 (4·15–16·99)	Fixed	Q=8·40, P=0·19, I^2=42·63
		Summary (6)	221 cases/1291 controls	5·91 (3·66–9·55)	Fixed	Q=7·51, P=0·19, I^2=33·42

Risk factor	Study Population	Study area (n of studies)	Cases/controls[d]	Odds Ratio, 95% CI	Model	Heterogeneity
	HBsAg− individuals excluding Wu et al (30)	Summary (5)	172 cases/769 controls	6·37 (3·74–10·86)	Fixed	Q=7·11, P=0·13, I^2=43·71
HBV only	General population	Summary (6) (5–8, 20, 30)	244 cases/1072 controls	11·2 (7·48–16·7)	Fixed	Q=2·37, P=0·80, I^2=0·00
	General population after adjusted heterogeneity	Summary (5) (5–8, 20)	171 cases/638 controls	11·3 (6·75–18·9)	Fixed	Q=2·36, p=0·67, I^2=0·00
Aflatoxin and HBV infection combined effects	General population	Summary (6) (5–8, 20, 30)	554 cases/1456 controls	54·1 (21·3–137·7)	Random	Q=13·65, p=0·02, I^2=63·36
	General population after adjust heterogeneity	Summary (5)(5–8, 20)	452 cases/847 controls	73·0 (36·0–148·3)	Fixed	Q=3·48, p=0·48, I^2=0·00

[d] If there was a series of follow-up studies in the same cohort need to be combined, only the numbers of cases and controls from the largest follow-up study were counted, although different odds ratios from different follow-up studies were combined to assess the effect. All the cases and controls were only counted once, and as well as in calculations presented in Table 4 and 5.

[e] This row shows the summary odds ratio of combing three follow-up studies in a Taiwan cohort in different years

[f] The summary odds ratio obtained for the Taiwan cohort was used to represent the effect of all studies in this cohorts, and combine with other studies

[g] Seven studies (7, 15, 17, 21–22, 25–26) reported adjusted ORs on aflatoxin-related HCC risk in HBsAg+ individuals. Four studies (5–6, 8, 16) (including two studies conducted in Sub-Saharan Africa countries) did not provide adjusted ORs directly, but provided data to calculate the unadjusted ORs. We calculated the unadjusted ORs for each of these studies and combined them with ORs from other studies with eligible data, thus we can include the effects of studies in Sub-Saharan Africa population. In subgroup analysis, the large variation of summarized ORs of aflatoxin-related HCC in HBsAg+ individuals may be explained by combining the unadjusted ORs. The heterogeneity was significant when studies were combined to examine the association between aflatoxin exposure and HCC risk in the general population and in HBsAg+ individuals.

[h] The summary odds ratio obtained from different follow-up studies for the Taiwan cohort was used to represent the effect of all studies in this cohorts, and combine with other studies

Table 3

Population attributable risk of liver cancer caused by aflatoxin exposure in HBV+ populations, HBV− populations, and the general population

Studies	Exposure measurement	PAR for aflatoxin attributable HCC risk in HBsAg+	PAR for aflatoxin attributable HCC risk in HBsAg−	PAR for aflatoxin attributable HCC risk in general study population adjusted by HBsAg+
Qian et al., 1994 (6) (Shanghai, China)	Multiple urinary aflatoxin metabolites	40% (24% – 47%)[i]	3.6% (0·3% – 5.6%)	9.0% (5.9%–10.4%)
Chen et al., 1996 (18) (Taiwan)	AFB_1 albumin adducts	n/a	n/a	10% (2.5% – 12%)
Chen et al., 1996 (19) (Taiwan)	AFB_1 albumin adducts Low vs undetectable	4.2% (0–13%)	n/a HBV individuals only	n/a HBV individuals only
	AFB_1 albumin adducts High vs undetectable	4.5% (0–11%)	n/a HBV individuals only	n/a HBV individuals only
		Sum = 8·7% (0–24%)	n/a HBV individuals only	n/a HBV individuals only
Wang et al., 1996 (7) (Taiwan)	AFB_1 albumin adducts	31% (0–51%)	0 (0–2.3%)	5% (0–11%)
	Urinary aflatoxin metabolites	41% (8.1%–54%)	1% (0 – 4.1%)	11% (1.4% – 13.7%)
Lunn et al., 1997 (20) (Taiwan)	AFB_1-DNA adduct	31% (0–75%)[j]	44% (29%–47%)	n/a
Yu et al., 1997 (21) (Taiwan)	1·61–2·85 ng/ml AFM_1 vs non-detectable)	2.1% (0%–7.2%)	n/a HBV individuals only	n/a HBV individuals only
	> 2·85 ng/ml AFM_1 vs non-detectable)	19% (2.2%–25%)	n/a HBV individuals only	n/a HBV individuals only
		Sum = 21% (2.2% – 32%)	n/a HBV individuals only	n/a HBV individuals only
Zhang et al., 1997 (22) (Henan, China)	Corn consumption	n/a	n/a	17·5% (8%–18·4%)
	Peanut consumption	n/a	n/a	36% (16% – 45%)
Kirk et al., 2000 (23) (The Gambia)	Ser 249 TP53 mutation	n/a	n/a	17% (12%–18%)
Omer et al., 2001 (24) (Sudan)	Average peanut butter consumption	n/a	n/a	23% (11%–29%)
Sun et al., 2001 (Taiwan) (25)	AFB_1 albumin adducts	12% (1.7% – 20%)	n/a HBV individuals only	n/a HBV individuals only
Ming et al., 2002 (26) (Qidong, China)	AFM_1	57% (16%–72%)[k]	n/a	n/a
Huang et al. 2003 (27) (Qidong, China)	Ser 249 TP53 mutation	n/a	n/a	17% (13%–18%)
Omer et al., 2004 (8) (Sudan)	Average peanut butter consumption	5.4% (0–62%)[l]	20% (9.0%–25%)	n/a
Kirk et al., 2005 (5) (The Gambia)	Ser 249 TP53 mutation	63% (39%–67%)[m]	12% (6.3%–17%)	13% (12%–14%)[6n]
Wu et al., 2009 (30) (Taiwan)	AFB_1 albumin adducts	3.7% (0–11%)	1.7% (0–4.6%)	2.1% (0.06%–3.4%)

Studies	Exposure measurement	PAR for aflatoxin attributable HCC risk in HBsAg+	PAR for aflatoxin attributable HCC risk in HBsAg−	PAR for aflatoxin attributable HCC risk in general study population adjusted by HBsAg+
	urinary aflatoxin metabolites	3.1% (0–11.7%)	4.7% (1.2%–6.7%)	4.4% (1.6%–6.3%)
Szymanska et al., 2009 (29) (Qidong, China)	AFB_1 albumin adducts	0 (0–14%)	n/a HBV individuals only	n/a HBV individuals only
Long et al., 2009 (28) (Guangxi, China)	AFB_1-DNA adduct medium vs low	n/a	n/a	6.8% (4.5%–8.5%)
	AFB_1-DNA adduct high vs low	n/a	n/a	19% (17%–20%)
	Total	n/a	n/a	26% (22%–29%)

[i] calculated from unadjusted OR

[j] calculated from unadjusted OR

[k] author estimated

[l] calculated from unadjusted OR

[m] calculated from unadjusted OR

[n] calculated from ORs unadjusted by HBsAg+)

Table 4

Estimated population attributable HCC risk from aflatoxin exposure in the general population by combining the eligible studies

Study population		Total exposed cases (n_1)	Total sample size (n_2)	P_c (n_1/n_2)	Summarized OR (95% CI)	PAR (95% CI)
General population adjusted by HBV status (6, 7, 18, 22–24, 27, 28, 30)	China	475	1588	0.299	5.99 (3.70–9.69)	25% (22%–27%)
	Taiwan	113	1102	0.103	2.01 (1.40–2.89)	5.2% (2·9%–6.7%)
	Sub-Saharan Africa	82	340	0.241	4.62 (2.12–10.08)	19% (13%–22%)
	Summary	670	3030	0.221	4·75 (2·78–8.11)	17% (14%–19%)
General population adjusted by HBV status after excluding Wu et al. 2009 (30)	Summary	583	2103	0.277	5.72 (4.42–7.40)	23% (21%–24%)

Table 5

Estimated population attributable HCC risk from aflatoxin exposure in HBV+ and HBV− populations by combining the eligible studies

Study population		Total HBsAg+ (or HBsAg−) (n_1)	Total HCC cases in HBsAg+ (or HBsAg−) (n_2)	Total exposed HBsAg+ (or HBsAg−) (n_3)	Proportion of HCC cases in HBsAg+ (or HBsAg−) (W_1)	Proportion of exposed HBsAg+ (or HBsAg−) (P_1)	Summarized OR (95% CI)	PAR (95% CI)
HBV+ population (5–8, 19–21, 25, 26, 29, 30)	China	457	189	276	0.414	0.604	2.00 (0.84–4.75)	16% (0–29%)
	Taiwan	564	254	314	0.450	0.557	1.81 (1.29–2.56)	14% (6.3%–21%)
	Sub-Saharan Africa	184	128	76	0.696	0.413	6.48 (0.22–194)	48% (0–69%)
	Summary[o]	1205	571	666	0.473	0.553	2.39 (1.50–3.82)	21% (10%–29%)
HBV+ population after excluding Szymanska et al. 2009 (29) and Wu et al (30)	China	208	63	108	0.303	0.519	3.01 (1.86–4.88)	16% (9%–20%)
	Taiwan	368	186	216	0.505	0.587	2.59 (1.63–4.13)	24% (14%–33%)
	Summary	760	377	400	0.496	0.526	2.90 (2.09–4.01)	25% (18%–30%)
HBV− population (5–8, 20, 30)	Taiwan	745	81	332	0.109	0.446	5.00 (2.22–11.28)	7% (3.8%–8·9%)
	Sub-Saharan Africa	513	122	113	0.238	0.220	8.40 (4.15–16.99)	15% (9.7%–19%)
	Summary	1632	227	617	0.139	0.353	5.91 (3.66–9.55)	8.8% (6.7%–10%)

[o]Studies (including two studies in Sub-Saharan Africa countries) with unadjusted ORs were also combined to calculate the overall PAR, thus the Sub-Saharan study population can be included.