Reduced Aflatoxin Exposure Presages Decline in Liver Cancer Mortality in an Endemic Region of China

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Abstract

Primary liver cancer (PLC) is the third leading cause of cancer mortality globally. In endemic areas of sub-Saharan Africa and Asia, PLC largely arises from chronic infection with hepatitis B virus (HBV) and ingestion of aflatoxins. Although synergistic interactions between these two risk factors have been observed in cohort studies in China, here we determined the impact of agricultural reforms in the 1980s leading to diminished maize consumption and implementation of subsidized universal vaccination against HBV in the 2000s on PLC primary prevention. A population-based cancer registry was used to track PLC mortality in Qidong, China and was compared with the timeline of HBV immunization. Randomly selected serum samples from archived cohort collections from the 1980s to present were analyzed for aflatoxin biomarkers. More than 50% reductions in PLC mortality rates occurred across birth cohorts from the 1960s to the 1980s for Qidongese less than 35 years of age although all were born before universal vaccination of newborns. Median levels of the aflatoxin biomarker decreased from 19.3 pg/mg albumin in 1989 to undetectable (<0.5 pg/mg) by 2009. A population attributable benefit of 65% for reduced PLC mortality was estimated from a government-facilitated switch of dietary staple from maize to rice; 83% of this benefit was in those infected with HBV. Food policy reforms in China resulted in a dramatic decrease in aflatoxin exposure, which, independent of HBV vaccination, reduced liver cancer risk. The extensive HBV vaccine coverage now in place augurs even greater risk reductions in the future. Cancer Prev Res; 6(10); 1038–45. ©2013 AACR.

Introduction

Primary liver cancer (PLC) is the third leading cause of cancer mortality worldwide with an estimated 696,000 deaths in 2008. Most PLC occurs in sub-Saharan Africa and southeast Asia; there are more than 370,000 deaths annually from PLC in the People's Republic of China alone (1,2). The major etiologic factors associated with PLC in China, as established from prospective cohort studies, are chronic infection with hepatitis B virus (HBV) and extended exposure to high levels of aflatoxin in the diet, especially from maize and peanuts (3). The largest study, comprising more than 18,000 men residing in Shanghai in the 1980s, examined HBV infection and aflatoxin exposure as independent and interactive risk factors for PLC (4). This nested case–control study revealed a statistically significant increase in the relative risk (RR) of 7.3 [95% confidence interval (CI), 2.2–24.4] for men who were chronically infected with HBV (hepatitis B surface antigen; HBsAg) but unexposed to aflatoxin. In men in whom urinary aflatoxin but not HBV biomarkers were detected, the RR was 3.4 (95% CI, 1.1–10.0). Furthermore, in men exhibiting both urinary aflatoxin biomarkers and positive HBsAg status, the RR was 59.4 (95% CI, 16.6–212.0; ref. 4). A subsequent cohort study in Taiwan confirmed these results (5). In light of this synergistic interaction, programs to eliminate either factor or both should have substantial impact on the burden of PLC.

Chronic infection with HBV has long been regarded as the major cause of PLC; thus, initiatives have been undertaken to implement universal immunization programs. More than 90% of the countries now routinely vaccinate newborns against HBV, and approximately 70% are now delivering three immunization doses (3). Newborns are targeted because HBV transmission often occurs from mother to child at birth or during the perinatal period. In 1986, Taiwan became the first region to vaccinate all newborns against HBV. Since then, the number of HBV carriers in the juvenile population has declined dramatically. As of 2009,
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The incidence of hepatocellular carcinoma was significantly lower among children aged 6 to 19 years in vaccinated versus unvaccinated birth cohorts (6). These results have heralded the expected benefits of global vaccination against HBV, projected as declines in hepatitis, cirrhosis, and PLC.

Qidong, China, located at the mouth of the Yangtze River, is an endemic area for PLC (7). HBV vaccination of newborns in the rural townships was initiated during the 1980s, but did not become universal until 2002. Qidong is a newly deposited river delta land unsuitable for the production of rice. Therefore, maize became the primary dietary staple and vector for aflatoxin exposure. Maize consumption in the 1970s, low socioeconomic status (i.e., occupation of "peasant"), and chronic infection with HBV were reported as major risk factors for PLC in neighboring Haimen County (8). In China, procurement practices tied rural household food consumption to local production, and in the commune system of the 1960s and 1970s, yields rather than quality were emphasized. Indeed, ducks and rats fed maize grown in Qidong in the 1970s exhibited very high incidence of liver cancer (7). Although China adopted institutional reforms after 1979 to shift from a planned economy to a market-oriented economy, importation from surrounding areas of foodstuffs, notably rice, was only permitted in rural Qidong beginning in 1985 (9). Rice typically harbors much lower levels of aflatoxin than maize. This study has delineated the changing dynamics of these key risk factors. Furthermore, we have examined the changes in PLC mortality by birth cohorts in Qidong in the context of two forms of primary prevention: HBV vaccination and reduction of dietary aflatoxin exposure.

Materials and Methods

Cancer registry

The Qidong Cancer Registry, a population-based registry that collects information on all deaths and all cancer cases in the county, was established by the Qidong Liver Cancer Institute (QDLCI; Qidong, Jiangsu, China) in 1972 (10). Demographic data of the county are provided by the household register office of the local security bureau, which tracks citizens by sex and place of residence yearly.

<table>
<thead>
<tr>
<th>Year</th>
<th>Village</th>
<th>Gender and age range</th>
<th># Screened</th>
<th>Random subsample: median age (IQR)</th>
<th># Assayed for aflatoxin biomarker</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>1982</td>
<td>“A,” Fusui, Guangxi</td>
<td>≥30–64</td>
<td>1,070</td>
<td>45.0 (38.0, 53.0)</td>
<td>77</td>
<td>15</td>
</tr>
<tr>
<td>1989</td>
<td>HeZuo, Qidong</td>
<td>≥30–59</td>
<td>494</td>
<td>45.0 (38.0, 54.0)</td>
<td>75</td>
<td>14</td>
</tr>
<tr>
<td>1995</td>
<td>Daxin, Qidong</td>
<td>≥30 and ≥25–65</td>
<td>1,006</td>
<td>44.3 (38.2, 50.8)</td>
<td>50, 50</td>
<td>11</td>
</tr>
<tr>
<td>1999</td>
<td>Daxin, Qidong</td>
<td>≥30 and ≥25–65</td>
<td>1,407</td>
<td>45.1 (38.1, 53.0)</td>
<td>50, 50</td>
<td>Not published</td>
</tr>
<tr>
<td>2003</td>
<td>HeZuo, Qidong</td>
<td>≥30 and ≥21–65</td>
<td>700</td>
<td>47.4 (39.3, 55.0)</td>
<td>50, 50</td>
<td>12</td>
</tr>
<tr>
<td>2009</td>
<td>HeZuo, Qidong</td>
<td>≥30 and ≥21–65</td>
<td>205</td>
<td>45.7 (38.7, 54.9)</td>
<td>50, 50</td>
<td>13</td>
</tr>
<tr>
<td>2012</td>
<td>Daxin, Qidong</td>
<td>≥30 and ≥25–65</td>
<td>206</td>
<td>47.3 (41.2, 54.0)</td>
<td>50, 50</td>
<td>Not published</td>
</tr>
</tbody>
</table>
likelihood function appropriately included the left censored observations corresponding to subjects with levels below the limit of detection (18). The generalized gamma distribution is characterized by three parameters: location (median), scale (interquartile ratio), and shape (tails; ref. 18) and extends the classical approach of using normal models for log-transformed biomarkers (corresponding to the case of the shape parameters being equal to zero). Maximum likelihood methods were used to obtain estimates of the parameters of the mixtures and to formally test for the significance of the mixture against simply one generalized gamma distribution, and the most parsimonious model was selected as the appropriate description of the biomarker distributions. To avoid overparametrization of mixtures when a substantial percentage of observations were below the limit of detection, we used mixtures of lognormal (shape parameter $\theta = 0$) and weibull (shape parameter $\tau = 1$) distributions.

To describe the benefit attributable to the reduction of aflatoxin exposure (defined as aflatoxin $> 0.5$ pg/mg albumin), we calculated the reduction of disease due to decreasing exposure from 100% (in the 1980s) to $p\%$ (more recently). Specifically, using 18% as the steady state of HBV positivity and among both aflatoxin unexposed and exposed individuals, the population attributable benefit (PAB) due to decreasing exposure to aflatoxin to $p\%$ from 100% is simply the differences in the rates of liver cancer relative to the rate when 100% were exposed. Namely, $\text{PAB} = (1 - p) \times [0.82 \times (3.4 - 1) + 0.18 \times (59.4 - 7.3)] / (0.82 \times 3.4 + 0.18 \times 59.4)$. The contribution to the PAB due to the aflatoxin reduction among the HBV negative is directly proportional to the first summand in the numerator $[0.82 \times (3.4 - 1)]$. In turn, the contribution to the PAB among the HBV positive is directly proportional to the second summand in the numerator $[0.18 \times (59.4 - 7.3)]$.

Results

Age distributions over time and liver cancer mortality in Qidong

Figure 1 shows the dramatic changes of the age distributions in this population rising from 17.0% over 50 years of age in 1973 to 22.7% in 1988 and 34.2% in 2003. These changes contribute significantly to the crude rates of PLC per 100,000 residents of Qidong rising slightly from 1972 through 1990 at levels around 50/100,000 before increasing in the 1990s to a rate of about 75/100,000. This changing distribution underscores the importance of age matching for the biomarker samples used in the study.

When mortality rates are examined within birth cohorts, there have been no significant changes in rates of mortality from PLC in Qidongese over the age of 40 over the past half century (Fig. 2A). In contrast, 60% to 75% declines are seen in 20 to 24, 25 to 29, and 30 to 34 year olds born in 1973–77 as compared with 1958–62; all are birth years in which no newborns would have been immunized with HBV vaccine. Declines continue to be seen in the birth cohort of 1983–87, where relatively few newborns would have been immunized (Fig. 2B).

Timeline for HBV immunization in Qidong

A pilot safety study was done in 1983–1984 in eight high-risk Qidong townships (out of 45) using the Merck HepB vaccine within 24 hours of birth, and at 1 and

![Timeline for HBV immunization in Qidong](image-url)
Mortality rates from PLC in younger birth cohorts in Qidong and throughout China, (19) number of newborns vaccinated against HBV (20). Vaccination rate in the rural areas was derived vaccine that was replaced by recombinant DNA produced by domestic plants, first providing plasma-

6 months after birth (19). In 1985, the program was expanded to 20 townships, and in 1987 to 26. Remaining townships were followed as control groups through 1990. Overall, about 97% coverage in the test townships was achieved in this limited vaccination program administered by the QDLCI (20). Figure 2B illustrates that 40% (40,605/102,566) of newborns in Qidong were vaccinated in the pilot study between 1983 and 1990. HBsAg prevalence in unvaccinated versus vaccinated children was 7.1% versus 1.7%, showing a 75% efficacy for this period (2). Given the low initial penetration into the population and the 75% efficacy of the vaccine, the likely impact of HBV immunization on PLC development in people born in Qidong before 1991 is modest. In 1992, in response to the recommendations of the World Health Organization, the Chinese government endorsed but did not subsidize universal vaccination. Vaccine was produced by domestic plants, first providing plasma-

Declining aflatoxin exposures
Annual surveys of aflatoxin contamination in maize in Qidong between 1973 and 1982 reported that 26% to 99% of acquired samples tested positive for aflatoxin at levels more than 20 ppb (7), the action level of the U.S. Food and Drug Administration. Average annual per capita maize consumption ranged from 82 to 124 kg during 1973–1982 (7); most Qidong families at that time consumed maize as a major dietary staple. In 1985, a sharp transition occurred because of the new open policy of provisionment in China (9). By 1998, only 9% of families in the Qidong area ate any maize; less than 1% ate 100 kg/year. Very little maize is consumed in the Qidong region in 2012. Conversely, the proportion of rural residents consuming some rice quickly reached 97.4% in 1986 and 99.2% in 1997 (24).

To describe accurately the aflatoxin exposures over the past quarter century, frozen serum samples collected in the years of 1989, 1995, 1999, 2003, 2009, and 2012 from residents of two rural villages in Qidong were retrieved from collection banks (Table 1). Previous studies have shown the aflatoxin–albumin adduct to be stable in frozen serum samples for at least two decades (16). Testing of the samples from studies spanning more than 20 years indicates there has been a dramatic decline in levels of exposure from the 1980s to the present. Figure 3 shows the raw data as well as the fitted generalized gamma distributions, whose appropriateness is supported by the good agreement between the observed and expected percentiles. As shown in Fig. 3,
median levels of aflatoxin–albumin adducts from residents of the villages of Daxin or HeZuo declined from 19.3 pg/mg albumin in 1989, to 3.6 in 1995, to 2.3 in 1999, to 1.4 in 2003, and undetectable (i.e., <0.5 pg/mg) in 2009 (median estimated at 0.2) and 2012 (median estimated at 0.06). Only 23% and 7% of serum samples had levels above 0.5 pg/mg in 2009 and 2012, respectively. All samples tested in the 1989 cohort were positive. There were no differences in aflatoxin exposures by gender. From these exposure levels in 2009 and in 2012, we estimated that of the observed reduction in PLC mortality (see formula in methods section), 65% (currently) and 78% (anticipated) is due to decreasing aflatoxin exposure among those infected with HBV. Because of the strong synergy between aflatoxin and HBV, the great majority [83% = (0.18 × (59.4 – 7.3))/0.82 × (3.4 – 1) + 0.18 × (59.4 – 7.3)] of this reduction was attributable to decreasing aflatoxin exposure among those infected with HBV.

Although remote from Qidong, Fusui County, Guangxi is another endemic area for PLC where maize was also the dietary staple (15). Figure 3 also presents the levels from older (1982) serum samples from that area. Levels of aflatoxin–albumin adducts were very high (median = 38.9 pg/mg albumin), and likely are representative of the levels also occurring in Qidong during the 1970s and early
80s when moldy maize was a significant part of the rural diet there as well (25).

Discussion

Changing patterns of cancer mortality, arising from population migration as well as implementation of screening, vaccination, and tobacco control programs, provide powerful evidence of underlying etiologies and efficacies of preventive programs. However, there are few cancer registries in low-income nations that can provide detailed time–trend data; so much of this knowledge is gleaned about cancers common to the most economically developed countries. The birth cohort data from the Qidong Cancer Registry clearly indicate dramatic, and HBV vaccination-independent, declines in mortality rates from PLC in younger adults over the last three decades. Moreover, as shown in Fig. 4, the age standardized (China) rate (CASR) of PLC mortality in Qidong exhibits an accelerating decline of nearly 45% since the early 1980s, reflecting a changing landscape of underlying risk factors. As validation of this registry, it, discouragingly, also presages the rising tide of cancers accompanying economic development as seen in other parts of the world; namely, breast, colon, and lung cancers (26).

HBV and aflatoxin exposure are key risk factors in this endemic area, whereas infection with HCV is not (27). Prevalence of infection with HBV is unchanged in Qidong adults to date (Fig. 4). Thus, the two- to three-fold decrease in PLC mortality seen between the birth cohorts of the 1960s and 1980s cannot be explained by differential HBV infections alone. Aflatoxin exposures, on the other hand, have been reduced dramatically—the generalized gamma distributions of aflatoxin–albumin adducts suggest upward of a thousand-fold reduction since the early 1980s (Fig. 3).

Nonetheless, risk factors for PLC other than aflatoxin need to be considered to explain the decline in PLC. Economic development has come only recently to Qidong. In 1990, less than 2% of rural households owned a refrigerator; by 2009 more than 50% did (28). However, declines in the CASR for PLC in Qidong during the 1980s indicate that underlying risk factors diminished at or before this time. Some epidemiologic studies have implicated drinking water obtained from ditches and ponds as an additional risk factor (9,29). Such water can be contaminated with microcystins, hepatotoxic peptides produced by algal blooms that may interact with aflatoxin to promote hepatocarcinogenesis. Exposures to microcystins were greatly reduced by the late 1970s through the efforts of the Qidong government to provide rural residents access to deep well water, which is largely devoid of microcystins (29). Improvements in quality of drinking water preceded the reduction in aflatoxin exposure by a decade, perhaps accounting for the initial decline in CASR.

This study provides evidence on PLC causation, that is, a drop in risk following a drop in aflatoxin exposure. We have shown here that reduction of aflatoxin exposure from 100% to 23% of samples positive for aflatoxin–albumin adducts resulted in an estimated PAB of 65% for reduction in the rate of PLC. Because of the strong synergy between aflatoxin and HBV, only 17% of the PAB was estimated to be due to the reduction of aflatoxin among those without HBV infection. Because of multiple sources of external data, it was not possible to calculate 95% CI of these effects.

In Taiwan, where economic development accelerated decades earlier than in Qidong, aflatoxin exposure also decreased. Liu et al. (30) have estimated that the population attributable risk in Taiwan for PLC due to aflatoxin exposure in HBV-infected populations has declined.

Figure 4. Dietary exposures, but not HBV carrier status, are associated with declining PLC mortality in Qidong. •, age standardized (China) rate of PLC in Qidong. —— prevalence of positivity for HBsAg in 30 to 34-year-old birth cohorts within two large-scale community-based screening studies conducted in Qidong in either 1976 or 2007–2009. HBV vaccination was not available to any newborns in these two birth cohorts. Median levels of aflatoxin–albumin adducts (shown in red) determined from the distributions presented in Fig. 4 from Qidong or (c) Guangxi. See text for details about the timelines for access to deep well drinking water and switch of dietary staple from maize to rice in Qidong.
from 31% in the 1980s to 12% in the 1990s and 3% in the 2000s. Perhaps the greatest needs for aflatoxin control are elsewhere. In Africa, maize is often used as the pioneer crop in newly deforested and developed lands. In several regions of Africa, maize has become, by far, the most important staple food, accounting for more than 50% of calories and up to 60% of field plantings (31). In 2004, one of the largest documented aflatoxin-poisoning outbreaks occurred in rural Kenya, resulting in 125 deaths. Aflatoxin-contaminated maize grown and consumed on family farms was the major cause. This outbreak marked the first time that biomarkers, namely aflatoxin–albumin adducts, were used to confirm the exposure in individuals (32).

There are multiple approaches to attenuating exposures to aflatoxins, including planting pest-resistant varieties of staple crops, reducing mold growth in harvested crops, improving storage methods following harvest, and using trapping agents that block the uptake of unavoidably ingested aflatoxins or agents that enhance its detoxication and elimination (33,34). Lower exposures to aflatoxin in North America and Europe result from dietary diversity, as well as regulatory actions governing allowable levels of aflatoxin in foods entering interstate and international commerce. All approaches could have usage in high-exposure areas. Nonetheless, the current results highlight the critical role of agricultural policies in reducing PLC risk in aflatoxin endemic areas.

PLC, like most chronic diseases, is multifactorial in origin. Although it is logical to assume that universal vaccination against HBV is poised to eliminate liver cancer (6,35), the attributive evidence supporting this conclusion is not so clear. The vaccination follow-up studies in Taiwan (6), a region where aflatoxin has also been shown to be a synergistic cofactor (5) and where exposures have likely dropped antecedent to or congruent with vaccination (30), does not yet provide definitive evidence of the unilateral success of HBV vaccination in cancer prevention. With synergy, primary prevention leading to attenuation of either the dietary carcinogen or the viral carcinogen, or both, could account for risk reduction. Clearly, the experience in Qidong shows that dietary change can affect rapid (within 1–2 decades), dramatic reductions in PLC mortality in cohorts of individuals not immunized against HBV. Certainly, HBV immunization is an essential effort for global prevention of liver disease but it will take more than a generation to reach full fruition of disease reduction, given the early age of viral transmission. Hence, prevention modalities for the 350 million HBV carriers (36) must not be neglected. Lack of a therapeutic vaccine against this virus, together with the world-wide poor prognosis for diagnosed patients with PLC, means that the public health community must consider additional approaches. Reducing dietary exposures to the environmental carcinogen aflatoxin, through a variety of strategies discussed above, is likely to significantly reduce liver cancer risk, even in those already infected with HBV.

Disclosure of Potential Conflicts of Interest
No potential conflicts of interest were disclosed.

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Writing, review, and/or revision of the manuscript: J.G. Chen, P.A. Egner, D. Ng, J.P. Jacobson, A. Muñoz, F. Wu, J.-M. Yuan, J.D. Groopman, T.W. Kensler
Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases): J.G. Chen, P.A. Egner, J.P. Jacobson
Study supervision: J.G. Chen, P.A. Egner, T.W. Kensler

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