

# Prevention of Hepatocellular Carcinoma: Vaccination and Dietary Changes Show Promise to Eliminate Most Cases of Human Liver Cancer

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## Introduction

Hepatocellular Carcinoma (HCC) is the 5<sup>th</sup> or 6<sup>th</sup> most common cancer worldwide. It has a poor prognosis, with the number of cases (626,000) closely resembling the number of deaths (598,000) making it the 3<sup>rd</sup> leading cause of cancer-related deaths in the world [1,2]. In the US, HCC is the 6<sup>th</sup> highest cause of cancer death in men and 9<sup>th</sup> highest in women. It has the second lowest 5 year survival rate (11.7%) of all cancers and its incidence shows a steady increase since 1980 [3]. Most cases of HCC occur in south-east Asia and sub-Saharan Africa, where dietary exposure to aflatoxin (AFB1), along with chronic hepatitis infection, are the most important risk factors [4-21]. Either AFB1 exposure or Hepatitis B Virus (HBV) carrier status alone increases the relative risk of developing HCC up to 17; when present together, the risk increases to between 60 and 70 [22-25]. The incidence of HCC in low and middle income countries is much higher than that of high income countries [26]. However, low levels of AFB1 contamination is widespread in the United States and may play a role in the etiology of HCC in the U. S [27,28]. Upon ingestion, AFB1 is activated by cytochrome P-450-mediated oxidation to mutagenic AFB1-8,9-epoxide (AFBO) [29,30]. AFBO binds to DNA, forms AFB1-N<sup>7</sup>-guanine adducts and produces mutations, including those observed in the p53 gene [31,32]. Protection against adduct formation occurs by detoxification of AFBO, principally by GST-mediated conjugation of AFBO with reduced glutathione (GSH) [29]. Unfortunately, humans do not have an effective GST detoxification subtype as does the adult mouse. HCCs associated with AFB1 and HBV have a high frequency of mutations in the p53 at codon 249 [9-11]. How binding of the AFB1 epoxide to DNA may cause this mutation is not known.

## Prevention

Two risk factors are responsible for the high incidence of HCC in resource-poor countries: dietary exposure to AFB1 and chronic HBV infection. The increasing incidence of HCC in Western countries is associated with HBV or Hepatitis C (HCV) infection. Global prevention of Hepatocellular Carcinoma (HCC) has become a possibility through elimination of aflatoxin exposure, immunization against hepatitis B [33-35] and control of Hepatitis C Virus (HCV) infection. However, each of these measures is costly and requires considerable public health efforts and liaison among healthcare providers and policy makers, as well as development of cost effective strategies applicable to local situations [26].

## Aflatoxin exposure

Only 7 or 8 years ago acute aflatoxin exposure caused over 150 deaths in Kenya [33]. Almost 30 years ago, Sun [15,16] documented the relationship between the source of water consumption and HCC in high incidence areas of China. This is because of the method of "curing" a major food product that is used in high-incidence areas of both China and Africa. The crop is "ground nuts" which are harvested in the form of entire plants, which are placed on the roof of houses to "cure" in the sun. High humidity in these areas promotes growth

of *Aspergillus flavus*, a mould that produces high amounts of aflatoxin [29]. Use of water collected from the roofs covered with moldy plants into rain barrels is associated with the highest incidence of HCC [15,16]; HCC incidence is next highest when ditch water from the fields is used. This is followed in order by use of river water and then well water (Figure 1). Since 1973, The Chinese government has been urging farming communities to drink only deep well water [16]. In Wuidong province, 80% of the population now drinks primarily well water as compared to only 20% previously. The workshop report of the Centers for Disease Control and Prevention and the World Health Organization produced a detailed report on what else should be done [33]. Steps are now directed toward managing the horticulture of the ground nut crop, including timing of planting, genotype of seeds used, irrigation, use of insecticides, timing of the harvest, how crop is dried and stored to prevent mould growth and treatment of the nuts after harvesting to decontaminate or inactivate the aflatoxin [36-38]. For example, pre-harvest practices such as proper irrigation or treatment with fungicides could be very effective and post harvest prevention of contamination may be accomplished by simple approaches such as drying the crops on cloth rather than on roofs or dirt, hand sorting to remove moldy plants, and storage in cold-dry places [38]. In one small area of China, reduction in aflatoxin exposure was achieved

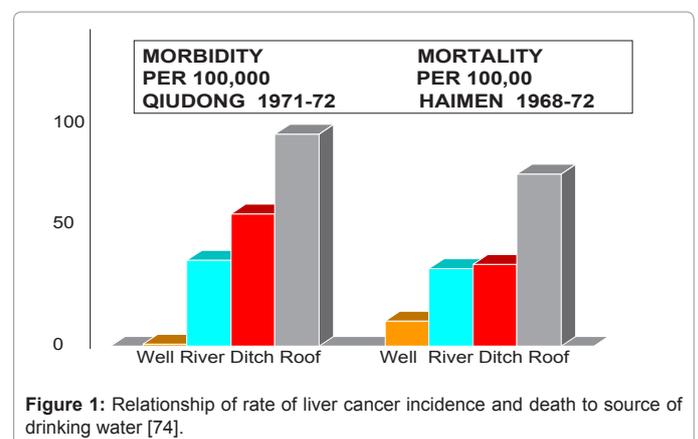


Figure 1: Relationship of rate of liver cancer incidence and death to source of drinking water [74].

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by switching from an *A. flavus* susceptible crop to a less susceptible crop: i.e., by a change from a ground nut based crop to rice [38]. It is recommended that this approach be combined with monitoring the food crop for aflatoxin contamination [34]. For example, monitoring of food products in Brazil revealed AFB1 contamination in 8.1% in peanuts and 6% in Brazil nuts [39]. Although the level of exposure was low and its effect problematical, continued surveillance of food products was recommended to try to reduce this exposure. Effective removal of contaminated foods, combined with selection of crops and use of optimal drying and storage practices, can essentially eliminate harmful exposure in developing countries [36,37]. However, developing countries may lack the resources and technologies required, as well as the infrastructure to effectively limit exposure. With rapid economic development this situation should not last much longer.

### Hepatitis B (HBV)

Vaccination against HBV infection protects against chronic liver disease which is a major risk factor for HCC [38,40]. In high risk HCC areas of the world up to 15% of individuals are chronically infected with HBV and up to 25% of these will develop HCC [41]. In these areas HBV infection almost always occurs in infancy either by passage from the mother during pregnancy or perinatally. Thus, to be effective any vaccination procedure needs to be directed to the mother or be administered neonatally. Ninety percent of those infected within the first year of life will develop chronic liver disease and eventually HCC [6]. Introduction of a vaccination program in Taiwan for all preschool children in 1984 has resulted in a marked decrease in the number of babies born to infected mothers and a 10 fold decrease in the rate of chronic HBV infection [42]. The occurrence of HCC among vaccines has already decreased by 70% as compared to non-vaccinated cohorts [43].

Because HBV vaccination should be administered neonatally to be most effective, it is advisable to administer Hyperimmune Globulin (HBIG) along with the vaccination [41]. This is because the infant's immune system may not be able to respond to the early vaccination. By administering HBIG, passive antibodies are able to check early virus infection until the recipients immune system can respond to the vaccine. Unfortunately, the expense of HBIG poses a problem for applying effective vaccination programs in developing countries. For programs now underway, it will take up to 30-50 years to determine how effective vaccination will be in reducing HCC. This is because there is at least a 20-30 year lag time between chronic liver injury and development of HCC [40]. For example a vaccination program in Gambia (West Africa) has shown 84% protection against HBV infection and 94% against chronic HBV carrier rates at 9 years of age [44]. However, because of the lag time mentioned above it will not be possible to evaluate the effectiveness on prevention of HCC until 2017 [45]. HBV vaccination has been recommended for all neonates in China since 1992 with essentially 100% coverage in Beijing being accomplished. The HBsAg carrier rate in the general population decreased to 7.2% in 2006 [46] and the prevalence of HBsAg decreased to 2.3% in children aged 5-14 years and to 1.0% among children younger than 5 years. Unfortunately even after administration of HBV immunoglobulin and HBV vaccine to children with HBsAg mothers the failure rate of HBV vaccination was 5-10% [46]. This failure is believed to be due to HBV S gene mutations and inadequate administration of the vaccine. In addition, the combined effect of vaccination and control of aflatoxin exposure has not been fully considered in these studies [47].

In chronically infected patients effective antiviral treatment using interferon or nucleoside analogues can delay disease progression to cirrhosis of HCC. A persistent high HBV DNA level is an accurate predictor for disease progression. Infected patients can be followed using ultrasonography and serum alpha fetoprotein every 3 months for early detection of HCC [48]. Early detection is critical to effective surgical treatment. Adjuvant interferon treatments may help prevent recurrence and nucleoside analogs may prevent *de novo* tumor development in the liver remnant by suppressing viral replication.

### Hepatitis C

Unfortunately, despite considerable effort, there is no effective vaccine for hepatitis C (HCV). The most effective approach to prevention of HCV are public health measures such as avoidance of high risk behavior (injected drug use) and promiscuous sexual activity [40]. In particular, only sterilized syringes and needles should be used and syringes should be disposed of after a single use. Chronically infected individuals should be identified and urged to try to avoid circumstances where infection might be transmitted. Non-infected individuals engaging in at risk behavior should receive counseling. In any case, hepatitis C is an increasing public health hazard in the Western World.

### Alcohol

In the United States and Europe, with a relatively low incidence of viral hepatitis, heavy ethanol consumption is also considered a major risk factor [49]. Only 9% of HCCs in the US are attributable to HCV and 20% to HBV [50]. The contribution of aflatoxin exposure to HCCs in the United States is not clearly known, but it is suspected that trace exposure to aflatoxin may synergize with alcohol injury and viral hepatitis as a critical risk factor for HCC [51,52]. Chronic ethanol intake causes chronic liver disease in humans, which progresses from early injury/steatosis, through hepatitis (fibrosis) to cirrhosis. The association of the late stages of cirrhosis with HCC has led most investigators to consider cirrhosis as a major risk factor for HCC [53-55]. However, there is actually a negative association between ethanol consumption and the risk of HCC in cirrhotic patients [56]. In addition, only one in 12 alcoholics develops cirrhosis [57] and only 5-10% of patients with cirrhosis develop HCC [58,59]. Although liver cirrhosis and development of HCC is associated with chronic alcoholism in humans, the majority of alcoholics do not develop cirrhosis and the percentage of alcoholics that develop HCC is low [60]. Probably not more than 10% of chronic alcoholics develop cirrhosis after prolonged chronic alcohol ingestion. In a series of 200 patients with chronic alcohol consumption periportal lymphocytic infiltration was identified in only 40% [61] and in a study of 268 patients with long term alcoholism, 151 had no significant fibrosis [62]. These findings suggest that risk factors other than cirrhosis determine which alcoholics will develop alcoholic hepatitis and HCC. It is clear that patients with a history of hepatitis B or C should limit alcohol intake, but it is not clear just how much restricting alcohol consumption would reduce the incidence of HCC.

### Summary

Current application of universal vaccination against hepatitis B and institution of effective measures to prevent aflatoxin contamination of grains should result in a massive reduction in the number of patients developing liver cancer in high incidence areas of the world within the next 20 years. A reduction should also become evident in lower incidence areas of the world, such as the US, but the effect is expected to be much less and more difficult to evaluate.

## Papilloma virus

Vaccination against hepatitis B may be considered the first and so far the most effective vaccine to reduce incidence of a cancer, but not the only one, as vaccination against Human Papilloma Viruses (HPVs) also promises to have a major impact on cervical cancer [63]. The vaccines now used for HPVs are directed to two major HPV types [16,18] and vaccination using this vaccine is projected to reduce human cervical cancer by 70% in the next 20 years [64,65]. Expansion of the vaccine to additional types [31,33,45,52,58] could reduce cervical cancer even more, up to 90% [63]. In addition, vaccination against Epstein-Barr Virus (EBV) for prevention of Burkitt lymphoma and against Human T-Cell Leukemia/Lymphoma Virus (HTLV) have been proposed [66] but to date there are no prospects of vaccines for these [67].

## Smoking

Despite these considerable accomplishments in cancer prevention, they pale in the face of the impact of smoking on lung cancer, cancer of the larynx, oral cavity, pharynx, esophagus, stomach, pancreas, liver, kidney bladder, uterine cervix and bone marrow, as well as colon, rectum and ovary [68,69]. Tobacco use is by far the largest single cause of premature death in the developed world accounting for up to 21% of deaths among men and 17% among women [70]. Although there are other hazards such as radon and occupational exposures, the vast majority of lung cancer deaths are attributable to cigarette smoking [71,72]. Although it is projected that smoking prevalence and smoking-attributed mortality will decrease in most developed countries [73], the rate of increased smoking in developing countries appears to be increasing as economic conditions improve. In any case, whereas major accomplishments in reduction of liver and cervical cancer have been made through vaccination, much more could be accomplished in cancer prevention by smoking reduction than by any other approach known at this time.

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