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Original Research

Aflatoxin B_1 exposure increases the risk of hepatocellular carcinoma associated with hepatitis C virus infection or alcohol consumption

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KEYWORDS
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Abstract ***Background:*** Hepatocarcinogenicity of aflatoxin B_1 (AFB_1) has rarely been studied in populations with hepatitis C virus (HCV) infection and those without hepatitis B virus (HBV) and HCV infection (non-B-non-C). This case-control study nested in a community-based cohort aimed to investigate the HCC risk associated with AFB_1 in HCV-infected and non-B-non-C participants.

Methods: Baseline serum AFB_1-albumin adduct levels were measured in 100 HCC cases and 1767 controls seronegative for anti-HCV and HBsAg (non-B-non-C), and another 103 HCC cases and 176 controls who were anti-HCV-seropositive and HBsAg-seronegative. Odds ratios (ORs) and 95% confidence intervals (95% CIs) were estimated using logistic regression.

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Results: In 20 years of follow-up, the follow-up time to newly developed HCC was significantly shorter in participants with higher serum AFB_1-albumin adduct levels in non-B-non-C ($p = 0.0162$) and HCV-infected participants ($p < 0.0001$). Within 8 years of follow-up, HCV infection and AFB_1 exposure were independent risk factors for HCC. Elevated serum AFB_1-albumin adduct levels were significantly associated with an increased risk of HCC newly developed within 8 years of follow-up in non-B-non-C participants with habitual alcohol consumption [crude OR (95% CI) for high vs. low/undetectable levels, 4.22 (1.16–15.37)] and HCV-infected participants [3.39 (1.31–8.77)], but not in non-B-non-C participants without alcohol drinking habit. AFB_1 exposure remained an independent risk predictor for HCV-related HCC after adjustment for other HCC predictors (multivariate-adjusted OR [95% CI], 3.65 [1.32–10.10]).

Conclusions: AFB_1 exposure contributes to the development of HCC in participants with significant risk factors for cirrhosis including alcohol and HCV infection.

1. Introduction

Liver cancer is the sixth most prevalent cancer in the world. Owing to the poor prognosis and a high mortality-to-incidence ratio of 0.95, it is also ranked as the second leading cause of cancer-related death worldwide with 745,000 deaths in 2012 (9.1% of total) [1]. Hepatocellular carcinoma (HCC) is the most common liver cancer (70%–90%) [2]. Viral hepatitis is the major risk factor for HCC. Approximately 80% of HCC cases are associated with chronic infection with hepatitis B virus (HBV) or hepatitis C virus (HCV) [3].

Aflatoxins are a group of naturally occurring mycotoxins produced by *Aspergillus* fungi [4]. Aflatoxin B_1 (AFB_1) is one of the most potent chemical liver carcinogens and also an important non-viral risk factor for HCC in humans [5,6]. A previous study estimated that about 5–28% of global HCC cases are attributable to aflatoxin exposure [7]. The major route of AFB_1 exposure is ingestion of crops such as corn, peanuts and rice. Liver is the primary target organ of AFB_1 toxicity. AFB_1 is preliminarily metabolised and activated by cytochrome P450 enzymes. The epoxidation of AFB_1 results in the highly active AFB_1 8,9-exo-epoxide which can covalently interact with DNA to form promutagenic AFB_1-N7-guanine adducts. AFB_1-N7-guanine adducts are released and subsequently excreted in the urine [4]. AFB_1 8,9-exo-epoxide can also bind to proteins including albumin to form AFB_1-albumin adducts [8].

AFB_1-associated risk of HCC has been primarily investigated in populations with a high prevalence of HBV infection [9]. Several cohort studies have demonstrated the strong synergistic interaction between chronic HBV infection and AFB_1 exposure [10–13]. The relative risk (RR) of developing HCC was much higher for individuals with both HBsAg seropositivity and detectable urinary levels of aflatoxin metabolites (RR [95% CI], 59.4 [16.6–212.0]) than those with detectable urinary levels of aflatoxin metabolites alone (RR [95% CI], 3.4 [1.1–10.0]) and those with HBsAg seropositivity alone (RR [95% CI], 7.3 [2.2–24.4]) when compared with those without these two risk factors as the referent (RR = 1.0) [13]. However, the hepatocarcinogenicity of AFB_1 has rarely been studied in populations with HCV infection and those without HBV and HCV infection (non-B-non-C).

Biomarkers of AFB_1 exposure include aflatoxin M_1, aflatoxin-mercapturic acid, and AFB_1-N^7-guanine adducts in urine as well as AFB_1-albumin adducts in serum [14,15]. Aflatoxin-DNA and aflatoxin-protein adducts are the products of damages to critical molecular targets, which may be used as markers for the biologically effective dose of aflatoxin exposure. In addition to a long half-life of 3-weeks, aflatoxin-albumin adducts in serum samples have also been shown to be stable after long-term storage (at least 25 years) [16], which makes aflatoxin-albumin adducts a suitable biomarker for epidemiological studies.

In Taiwan, the estimated prevalence of antibody against HCV (anti-HCV) is 4.9% in the general population [17,18]. We enrolled a Community-based Cancer Screening Project (CBCSP) cohort in 1991–1992, and all the participants were examined for their serostatus of HBV surface antigen (HBsAg) and anti-HCV. In addition, participants were followed up for newly diagnosed HCC until 2011. In this case-control study nested in the CBCSP cohort, baseline serum AFB_1-albumin adduct levels were measured to evaluate the AFB_1 exposure in HBsAg-seronegative participants. We aimed to investigate the impact of AFB_1 exposure on the onset time and risk of HCC in HCV-infected and non-B-non-C participants.

2. Materials and methods

2.1. Study cohort

Study participants were selected from the CBCSP cohort established in 1991–1992. A total of 23,820 residents