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Hepatitis B virus infection contributes to oxidative stress in a population exposed to aflatoxin B1 and high-risk for hepatocellular carcinoma

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Abstract

Biomarkers of Hepatitis B Virus (HBV) infection, aflatoxin B1 (AFB1) exposure and oxidative stress were detected in 71 hepatocellular carcinoma (HCC) patients and 694 controls from southern China. Plasma level of AFB1-Albumin-Adducts (AAA) and protein carbonyl content (PCC) were significantly higher in the 71 HCC cases than in any age/gender matched HBV sero-status groups (P<0.001). HCC patients positive for the *p53*-249 G-T mutation had a marginally higher level of PCC than those negative for the mutation (p=0.077). HBV infection had a prominent influence on the association between AFB1 exposure and oxidative stress biomarkers in the controls. Our study indicates a significant contribution from HBV infection to oxidative stress in a population with AFB1 exposure which might substantially increase risk for HCC in this region.

Keywords

HBV; Aflatoxin; oxidative stress; hepatocellular carcinoma

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Introduction

Hepatocellular carcinoma (HCC) ranks among the 10 most common malignancies worldwide, accounting for 5.6% of all human cancers [1,2]. The broad characteristics of HCC can be partially explained by the patterns of exposure to the key risk factors in different regions [2]. In Asia and Africa where the majority of cases live, aflatoxin and hepatitis viruses (HBV and HCV) are important factors giving rise to the extraordinarily high incidence rates (24.2~35.5/100,000) of HCC in these areas [1,2]. HBV-induced chronic active hepatitis and cirrhosis constitute major risk factors in liver carcinogenesis [3]. HBV is associated with 70~75% of all HCC cases in Asia, the continent with the highest prevalence of HBV [4]. Also, the role of dietary exposure to aflatoxin B1 (AFB1), a category I known human carcinogen and a potent genotoxic agent, in the development of HCC has long been documented in many model systems (reviewed in [5-7]). A synergistic effect of AFB1 and HBV on HCC risk has been reported in many studies (reviewed in [5,8]).

Reactive oxygen species (ROS) are potential carcinogens because of their roles in mutagenesis, tumor promotion, and progression [9]. ROS and oxidative damage have been shown to contribute to the genotoxicity of AFB1 [6]. AFB1 is metabolized by constitutive cellular enzymes, during which there is formation of free radicals [10]. The process results in both lipid peroxidation and covalent adducts with DNA and proteins [6]. A strikingly high frequency of G-T transversions at the third base of codon 249 in *p53* was found in Asian and African HCCs with high level exposure to both HBV and AFB1 [5,6]. In vitro studies suggest that the hot-spot mutations may form through preferential binding of AFB1 as well as AFB1-induced oxidation at CpG sites [11,12]. Extensive oxidative DNA damage had also been observed in hepatocytes of HBV-positive, transgenic mice [13] and HBV-infected humans [14,15]. Oxidative stress in the pathogenesis of hepatitis virus might be caused by a combination of chronic inflammation, iron overload, liver damage, and proteins encoded by the virus [16]. This might in consequence, increase sensitivity to chemical carcinogens and is probably relevant in humans exposed to both hepatitis viruses and chemical carcinogens [3]. However, direct evidence linking the two carcinogens to a common damage endpoint is rare, especially in population studies. We hypothesize that if there is a carcinogenic effect of oxidative stress leading in the development of HCC, it would be most evident in a population exposed to environmental hepatocarcinogens where exposure is strong enough to manifest the mutagenic effects. To test this hypothesis, we conducted a multi-center collaborative study in a Chinese population at high-risk for HCC, using multiple biomarkers to profile oxidative status.

Materials and methods

Samples collection

Study subjects came from Guangxi Zhuang Autonomous Region, China, a region of elevated HCC risk and AFB1 exposure and HBV infection [17,18], and Chengdu City, Sichuan province, a region with a relatively low incidence of HCC. Details on recruitment have been described elsewhere and AFB1-Albumin-Adducts and Protein carbonyl content data on a subset of 404 subjects in this analysis has been reported previously [19]. In brief, a total of 695 healthy subjects (530 males, 165 females, age 36.6 ± 15.6 yrs) were recruited from three regions of P.R. China. The local cancer registry revealed a gradient in HCC mortality for males in these regions (Fusui 92-97/100,000, Nanning 32-47/100,000 and Chengdu 21/100,000, respectively) [18]. Samples were collected in the morning before daily work in turn from Chengdu (n=119), Nanning (n=486) and Fusui (n=90) during April to June, 2001. Subjects were screened for hepatitis virus (HBV, HCV, HDV, HEV and HGV) serology and those positive for any hepatitis virus except HBV were excluded from this study. Liver biochemistry (alanine aminotransferase (ALT), aspartate aminotransferase (AST), albumin (ALB), globulin (GLO) and plasma total protein (TP)) was quantified for all subjects as described previously [18]. All liver

biochemistries were determined on a Beckman LX20 Chemistry Analyzer (Beckman Coulter, Inc., Brea, CA, USA) in the same laboratory. One subject from Chengdu and one from Nanning City had extraordinarily high concentrations of AAA (5751 fmol/mg albumin) and carbonyl (1.4 nmol/mg protein), respectively. The former was confirmed to be infected with tuberculosis in a later follow up. These two subjects were excluded from the analysis. The 71 HCC subjects were a subset of 561 patients from Guangxi and hospitalized based on national diagnostic criteria for HCC between December, 1998 and July, 2005, and confirmed by pathology of the specimens from biopsy or operation. To compare AAA and PCC levels, they were selected by matching age and gender with 71 controls in each of the four HBV sero-states categories (stated below) from the same region. Plasma was collected right after admission and 37 tumor tissues were collected during surgery. Informed consent was obtained from each participant before sample collection.

Enzyme-linked-immunosorbent-assay (ELISA) for concentrations of AAA and PCC in plasma and AFB1 and 8-OHdG in urine

AFB1-Albumin-Adducts (AAA) and Protein carbonyl content (PCC) were quantified for all 71 HCC cases and 695 controls. AAA concentrations in plasma were quantified as described previously by competitive ELISA using a polyclonal antiserum that recognizes the AFB1-lysine adduct in digested albumin [20]. Values were normalized to the amount of albumin (fmol/mg albumin) and further by albumin concentration in plasma (fmol/ml plasma). The minimum level of AAA among these subjects was 32.6 fmol/mg albumin, above the limit of sensitivity of this assay (10 fmol/mg albumin) and quality control measures were taken as described previously [20]. PCC was determined by a noncompetitive ELISA as described previously that involves derivatization of carbonyl groups with dinitrophenylhydrazine (DNP) followed by detection of the DNP group with a commercial antibody [19]. PCC values were first normalized by TP (nmol/mg TP) and then normalized by TP concentration in plasma (nmol/ml plasma). The range of PCC in these subjects was between 0.11 - 1.41 nmol/mg TP, within the linear range of this assay (0.1 – 2.5 nmol/mg TP) [21], and comparable to the reported level of PCC in human plasma (0.4 – 1.0 nmol/mg TP) [22]. The intra-assay variation was 10.0% (n=22), similar to the mean variation of ±8.8% reported by Buss et al. [21] AFB1 and 8-hydroxydeoxyguanosine (8-OHdG) concentrations in urine among 290 adults in Nanning were quantified by competitive ELISA as described previously using monoclonal antibodies AF8E11 and 1F7, respectively [23,24]. Values were normalized to the urinary concentration of creatinine (fmol AFB1/mg creatinine and ng 8-OHdG /mg creatinine).

P53 gene codon 249 mutation in HCC tumors

Tumor tissues were digested by proteinase K (Qiagen Inc., Valencia CA USA) and DNA was extracted by phenol-chloroform. *Hae*III was from New England BioLabs Inc. (Ipswich, MA USA) and the PCR-RFLP assay for the *p53* codon 249 mutation was carried on as reported previously [25] and confirmed by sequencing (Alpha Biolaboratory Inc. Burlingame CA, USA).

Statistical analysis

Liver function indices and protein adducts levels were compared among the 71 HCC cases and four age and gender matched groups of HBV-status controls (71 in each category). Associations among biomarkers in plasma (liver function indices, AAA and PCC) and urine (AFB1 and 8-OHdG) were analyzed in controls. A logarithmic transformation of AAA, PCC, urinary AFB1 and 8-OHdG and liver function indices (TP, ALB, ALT and AST) was used for Oneway ANOVA and linear regression analysis. Spearman correlation was used to examine the association among biomarkers and Mann-Whitney Test was used to compared the protein adduct levels between *p53* codon 249 mutation carriers and non-carriers. For clarity of

presentation, the untransformed adduct levels and SD are presented throughout the text and figures. The relationship of adduct levels to risk factors was examined in a linear regression model or GLM model. All statistical analyses were performed using SPSS 10.0 software (Chicago, USA). A two-tailed p value <0.05 was considered significant.

Results

Plasma biomarkers

HBV serology in HCC cases and controls—25.1% (174/694) of the normal population and 100% (71/71) of the HCC cases were HBV (+), defined as positive for any one of the markers (sAg/eAg/eAb/cAb). The positive percentages for sAg/sAb/eAg/eAb/cAb were 10.6%(75/694), 53.9%(380/694), 1.1%(8/694), 14.0%(99/694), 22.4%(158/694) for the controls and 93.0%(66/71), 1.4%(1/71), 11.3%(8/71), 73.2%(52/71), 98.6%(70/71) for the HCC cases, respectively. Among controls, the 8 subjects positive for eAg were all in the sAg (+) group, and 87.9% (87/99) of the subjects positive for eAb were concomitantly positive for cAb, so the above two markers were combined into the sAg(+) and cAb(+) categories, and the HBV sero-status of the controls were categorized into four groups: **null** (null for all HBV markers, 30.6%, 212/694), **sAg(+)** (HBsAg(+) regardless of sAb or cAb status, 10.8%, 75/694), **sAb(+)** (sAg(-)sAb(+)cAb(-), 46.0%, 319/694), and **cAb(+)** (sAg(-)cAb(+), 12.5%, 87/694).

Comparison of protein adducts levels among subjects from the three regions—Similar to our previous analysis of a smaller sample size [19], there were significant differences in AAA and PCC levels among the populations from the three different regions, in accordant with their HCC incidences (data not shown).

Comparison of protein adducts levels among 71 HCC cases and age and gender matched HBV-status-controls—In contrast to all HBV sero-status control groups (71 age and gender matched controls in each category), there were significantly lower levels of TP, ALB and GLO in HCC patients (P<0.01) (Figure 1). Among controls, HBsAg(+) subjects had significantly lower concentrations of albumin (P<0.05) but significantly higher levels of ALT and AST (P<0.001) (data not shown). These results suggested that protein concentration is an important issue to be considered when comparing protein adducts levels among subjects with different HBV infection status.

HCC patients had significantly higher levels of AAA and PCC than any matched HBV status group of controls (P<0.001), regardless of plasma protein concentrations. Among the entire control group (n=694), the difference in protein adducts levels was only significant for PCC between the sAb(+) and HBV-null groups (P=0.030), suggesting the presence of HBsAb might be a protective factor, associated with lower PCC levels. Interestingly, the increment in PCC per fmol of AAA was significantly higher in HCC cases, followed by the sAg(+) and cAb(+) control groups, and the sAb(+) control group, suggesting a distinct stress response to AFB1 exposure by HBV status (Figure 2).

In the 37 HCCs with available tumor DNA, the frequency of *p53* codon 249 mutations (all were AGG-AGT transversions) was 64.9% (24/37). HCC patients positive for *p53*-249 mutations had a marginally higher level of PCC (21.6 nmol/ml plasma) than those negative (15.9 nmol/ml plasma) (P=0.077, Mann-Whitney Test) but no difference in AAA levels (p=0.456).

The present classification may not completely specify HBV infection sero-status. For example, the IgG and IgM forms of cAb were not distinguished in the present study. Among the 87 sAg (-)cAb(+) subjects, those eAb(+) had similar levels of AAA yet significantly (t=3.285, p<0.001) higher level of PCC than those eAb(-), suggesting a different status of oxidative stress

in this category (data not shown). If HBV active infection is defined as positive for sAg, eAg, eAb or cAb together with ALT >40 IU/ml and/or AST >45 IU/L, subjects with active HBV infection had a significantly higher level of AAA (12.1±6.1 pmol/ml, n=39) than non-active HBV infectants (9.0±5.8 pmol/ml, n=133, p=0.002) and HBV-free subjects (9.3±6.8 pmol/ml, n=518, p=0.001).

Association between AAA and PCC in controls—AAA level was correlated with PCC in the 693 controls (r=0.155, P<0.001). In addition, HBV infection status influenced this association: the association between AAA and PCC was significant among sAb(+) (n=319, r=0.309, P<0.001) and cAb(+) (n=87, r=0.310, p=0.004) control groups, marginally significant in the HBV-null (n=212, r=0.133, P=0.055) but not in the sAg(+) control group (n=75, r=0.029, P=0.807). (Figure 3)

Association between protein adducts and amino transferases (ALT and AST) in controls—PCC was associated with ALT and AST levels in all HBV sero-status groups (r=0.166~0.234, P<0.05). AAA was associated with AST levels in only sAg, eAg, eAb, or cAb (+) subjects (r=0.197, P=0.010).

Association between protein adducts and HBV sero-status in 693 controls—In a linear regression analysis, if single HBV markers were used, AAA (P=0.002), sAb (P=0.050), eAb (P=0.009) and cAb (P=0.019) significantly contributed to PCC levels. And if HBV sero-status category was applied, AAA (P=0.022), HBV status (P=0.005), gender (P=0.056) and age (P=0.001) were factors significantly contributing to PCC levels.

Urinary biomarkers

Association among plasma and urinary biomarkers of AFB1 and oxidative stress (290 adults in Nanning, China)—Urinary excretion of AFB1 was associated with both the AAA (r=0.190, p=0.001) and PCC (r=0.176, p=0.003) levels in plasma, suggesting an association between recent AFB1 exposure and oxidative protein damage. Urinary excretion of 8-OHdG was also associated with urinary AFB1(r=0.433, p<0.001) and interestingly, in a linear regression, urinary 8-OHdG was associated with plasma AAA level (p=0.011), when urinary creatinine was an independent factor in the model. This suggests an association between recent and chronic AFB1 exposure and oxidative DNA damage.

Furthermore, this association differed by HBV categories. The association between urinary AFB1 and 8-OHdG was consistently significant in all HBV sero-status groups (r=0.305, p=0.031 in HBcAb(+), r>0.400, p<0.001 for the other categories), and the association between urinary AFB1 and plasma PCC was consistently significant in all (r>0.260, p<0.05) except the HBV-null group (p=0.595). This might suggest significant contributions to oxidative DNA and protein damage from recent AFB1 exposure. The association between plasma AAA and urinary 8-OHdG (r=0.446, p=0.020), between plasma PCC and urinary 8-OHdG (r=0.4486, p=0.019), was significant only in sAg(+) subjects, suggesting profound oxidative DNA damage from chronic AFB1 exposure and carbonyl stress among HBV carriers. Finally, the association between plasma AAA and urinary AFB1 was significant in the HBV null group (r=0.256, p=0.011) and marginally significant in the HBsAb(+) group (r=0.163, p=0.088) but not in the sAg(+) and cAb(+) groups, suggesting that HBV infection could interfere with the metabolism and subsequent excretion of AFB1. (Figure 3)

Comparison of urinary excretion of AFB1 and 8-OHdG among HBV status groups (290 adults in Nanning)—The HBcAb(+) group had significantly higher levels of urinary AFB1 and 8-OHdG than the HBV null and sAb(+) groups (p<0.05). There were no significant associations between urinary biomarkers and either ALT or AST, but among

subjects with ALT< 41IU/L and AST< 46IU/L, there were significantly ($p<0.05$) higher levels of urinary AFB1 and 8-OHdG in HBV(+) subjects (n=66) than the HBV(-) (n=181), suggesting higher level of AFB1 exposure and oxidative DNA damage in HBV infected subjects in this sub-set (data not shown). (Figure 4)

In a GLM model, urinary AFB1 ($p=0.002$), HBV sero-status ($p=0.010$), the interaction of HBV and urinary AFB1 ($p=0.010$) were independent predictors of PCC, whilst urinary AFB1 ($p<0.001$), age ($p=0.004$), chronic diseases ($p=0.004$) and the interaction between PCC and HBV status ($p=0.063$) were independent predictors of urinary 8-OHdG in this population. Figure 5 summarizes the associations among the oxidative stress, HBV and AFB1 biomarkers in this population.

Discussion

The frequencies of HBsAg(+) in our controls (10.6%) and HCC cases (93.0%) are similar to those found 20 yrs ago in this region [26,27], suggesting that even after decades of nationwide vaccination, HBV infection is still a major risk factor for HCC in this area as well as in the country [28]. It is not surprising to see a significantly lower concentration of plasma proteins in HCC cases and HBsAg(+) control subjects. However, this leads to concerns on protein adduct dosimetry in these special populations [19]. Determining the influence of protein concentration on protein adduct dosimetry in a mal-nourished animal model might provide supportive data addressing this issue.

This study highlights the necessity of using multiple markers rather than a single HBV marker to define individual infection status. The host immunoresponse to HBV is a dynamic process [29]. Our data revealed that HBV status, defined by either a combination of HBV sero-markers and/or amino transferases, had a profound influence on host response to AFB1 in terms of AFB1 metabolism and oxidative damage to protein and DNA. In fact, HBsAg plus aminotransferase or the pattern of HBV sero-transversion has been used to differentiate active or past HBV infection, and to explore the association between active HBV infection and AAA levels [30,31]. One limitation of the present study is the IgM form of cAb, a marker of recent HBV infection [29], was not determined. Among the 87 sAg(-)cAb(+) subjects, those positive for eAb had similar levels of AAA yet significantly higher level of PCC than those negative, suggesting a subset in this category possessed higher oxidative stress relevant to HBV replication. The different redox-status among HBV-null, sAg(-)cAb(+) and HBsAb(+) individuals we observed might be one explanation for the etiology of HBsAg(-) HCC but the mechanism remains unknown [3]. To address this limitation in our ongoing study, HBV DNA copy number and quantified/dynamic HBV sero-markers are being measured to distinguish a possible subset of HBV infectants susceptible to oxidative damage.

In this population, sAb(+) subjects had significantly lower levels of plasma AAA and PCC and urinary AFB1 and 8-OHdG than those of cAb(+), suggesting a protective effect from HBsAb in reducing oxidative damage from AFB1 exposure. This supports the role of HBV vaccination in preventing HCC in HBV endemic areas [32]. Strikingly, if AFB1 exposure was balanced, the order (from high to low) in the increment of PCC per fmol of AAA in age and gender matched groups was HCC > cAb/sAg > null > sAb (Figure 3), suggesting that HBV infection alone significantly increased oxidative stress. Oxidative damage to proteins may be a critical pathological event because enzyme inactivation can have rapid effects, by nature of their catalytic functions [33]. PCC (aldehyde or ketone) is a widely used marker for the presence of oxidative stress in physiological and pathological conditions [34]. It is hypothesized that oxygen uptake changes due to the viral infection process is responsible for the increase of protein oxidation and the death of virally infected cells [35]. This is supported by the observation of a higher risk of aminotransferase flare-up among symptom-free HCV carriers

with impaired redox state [36], but seems not in accordance with the consistent associations we observed between PCC and ALT and AST levels in all HBV sero-status groups.

High level of dietary aflatoxins in Guangxi, an HCC endemic area in China, was first reported in the 1980s [27]. We found in HCC patients from this region extraordinarily high levels of AAA, a biomarker of chronic AFB1 exposure [20], and a high frequency (64.9%) of *p53* codon 249 mutations, a molecular fingerprint of AFB1 contamination in tumor tissues [5,6]. The significant correlation between PCC and both plasma AAA and urinary AFB1, to the best of our knowledge, has not been reported previously. Also, the AAA and PCC levels in people from the three regions were in accordance with the corresponding HCC incidences. This is consistent with previous reports [37,38] and a recent investigation on food hygiene in this area [39], supporting the role of AFB1 exposure as an independent risk factor for HCC in this area [37]. Moreover, this suggests that even after decades' long implementation of a primary prevention program for food hygiene improvement, AFB1 contamination is still a prominent risk factor for HCC in this region.

In contrast to AAA which reflects exposure over a period of weeks/months because of the relatively long half-life of albumin (~20 days) in humans [40], urinary excretion of aflatoxin metabolites is very rapid and hence is dependent on the consumption of aflatoxin in the proceeding 24 hrs [41]. The significant associations between urinary AFB1 and urinary 8-OHdG, and between urinary AFB1 and PCC in this study suggests that urinary excretion of 8-OHdG as a biomarker of DNA damage, and PCC as a biomarker of protein damage, may partly result from the recent exposure to AFB1. This is a strong evidence supporting the reported association between levels of urinary AFB1 metabolites and HCC risk [23,42-44]. 8-OHdG is widely studied and considered a key biomarker of oxidative DNA damage [45,46]. We found significant associations between urinary 8-OHdG and urinary AFB1 and plasma AAA in a linear regression model, suggesting associations between recent and chronic AFB1 exposure and oxidative DNA damage. Shimoda et al. reported a significant correlation between the 8-OHdG content in noncancerous liver tissues with individual serum ALT concentration [47]. However, we saw no direct association between the urinary 8-OHdG and aminotransferase levels. Given the uncertainty of in vivo 8-OHdG turnover, and the multi-source contribution to the urinary excretion of 8-OHdG, to what extent its level represents events occurring inside the liver, the target organ for both HBV and AFB1, remains unclear.

A synergistic interaction between AFB_1 exposure and HBV infection on HCC risk has been proposed in epidemiological [23,40] and animal studies [48-50]. However, elevated levels of AAA in HBV(+) adolescents and children had been reported in our study conducted in Taiwan [51] and three other studies in Gambia [31,52,53] but not in adults [30,54]. Kew summarized a number of possible mechanisms for this synergistic effect, mainly focused on the devastated effect from HBV infection to the AFB1 exposed subjects (review in [8]). However, new evidence suggests that the interaction between HBV and AFB1 might not always be in one direction: carcinogen-induced transcription factors may influence viral carcinogenesis and initiate HCC [55]. AFB1-induced immunosuppression might increase the risk of secondary infection [56]. Further, recent DNA damage sites can serve as sites for hepadnaviral DNA integration, and increasing the number of DNA damage sites dramatically increases viral integration frequency [57,58].

The present data highlights a synergistic effect of HBV and AFB1 in inducing oxidative stress and subsequently increasing risk for HCC in this region. First, there was interference from HBV infection on the metabolism and subsequent excretion of AFB1 (plasma AAA *vs.* urinary AFB1). Second, there was a protective effect from HBsAb in reducing oxidative damage from AFB1 exposure whilst HBV infection altered individuals' response to external and internal carcinogens: HBV infection enhanced the effects of exposure to AFB1 (active HBV infection

vs. AAA), enhanced AFB1-relevant damage to parenchyma cells in liver (AAA *vs.* AST), and enhanced oxidative response to AFB1 exposure (PCC/AAA *vs.* HBV, AAA *vs.* 8-OHdG, and PCC *vs.* 8-OHdG). Third, HCC patients positive for *p53* codon 249 mutations had a similar level of AAA but a higher level of PCC which supports the mutagenic role of ROS [59]. And finally, the interaction of HBV and urinary AFB1 was an independent predictor of PCC in a multivariate model. These observations suggest an essential role for oxidative stress in hepatocarcinogenesis with connections to both HBV and AFB1. An antioxidant-based chemoprevention strategy might be appropriate for individuals presently at risk in this area [60]. In fact, chemoprevention trials conducted in this region have shown promising effects: Oltiplaz inhibited phase 1 activation while increasing phase 2 conjugation of aflatoxin [61], and an intervention by green tea polyphenols significantly decreased urinary 8-OHdG levels [62].

In summary, we evaluated a biomarker profile of oxidative stress in a population exposed to environmental carcinogens, highlighting the contribution from HBV and aflatoxins, and especially the synergistic effect of both on macromolecular damage and risk for HCC in the region. The clear associations between oxidative biomarkers and exposure to HBV and AFB1 in this population suggest a core role of oxidative damage to macromolecules in HBV and AFB1 relevant hepatocarcinogenesis [6].

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgements

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Abbreviations

HBV

Hepatitis B Virus

AFB1

aflatoxin B1

AAA AFB1-albumin adduct

PCC Protein carbonyl content

HCC hepatocellular carcinoma

ROS Reactive oxygen species

ALT alanine aminotransferase

AST aspartate aminotransferase

ALB albumin

GLO globulin

TP total protein

ELISA enzyme-linked-immunosorbent-assay

8-OHdG 8-hydroxydeoxyguanosine

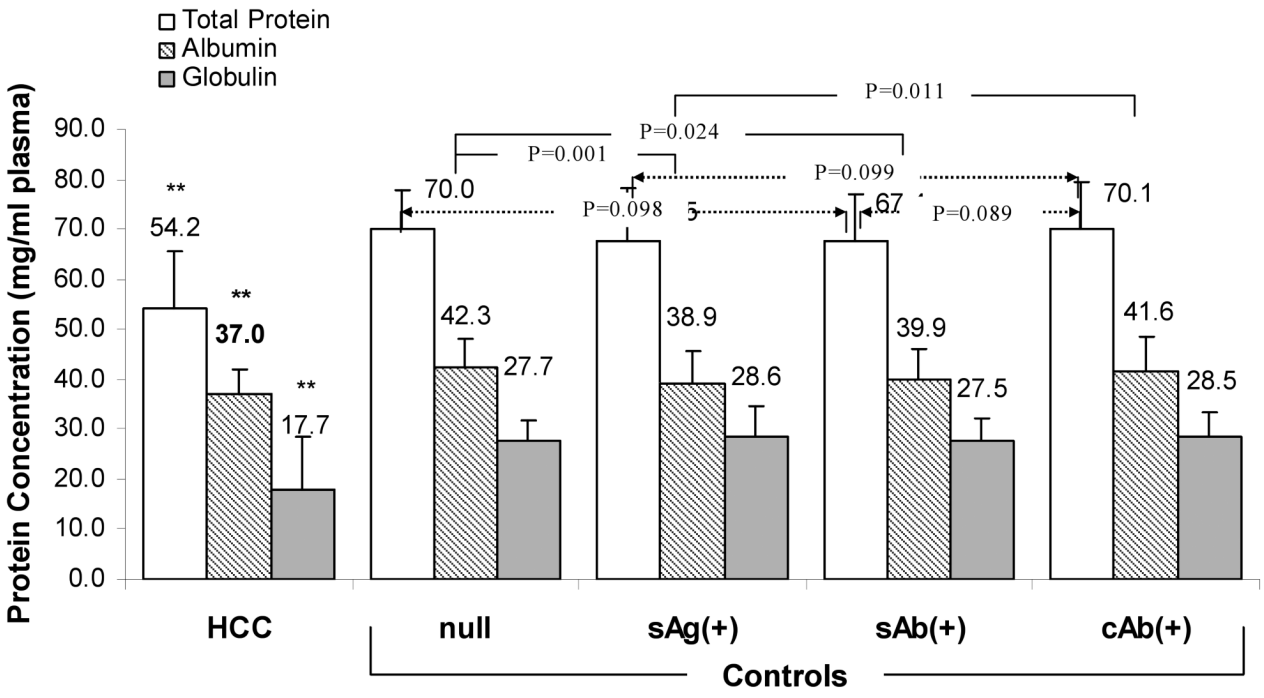

Figure 1. Comparison of Protein Concentration among age and gender matched HCC and HBV sero-status groups (n=71 per group)

** Levels of TP, ALB and GLO in HCC patients were significantly lower than any HBV sero-status group (P<0.01). Only p values less than 0.1 are indicated. Null (null for all HBV markers), sAg(+) (HBsAg(+) regardless of sAb or cAb status), sAb(+) (sAg(-)sAb(+)cAb(-)), and cAb(+) (sAg(-)cAb(+)).

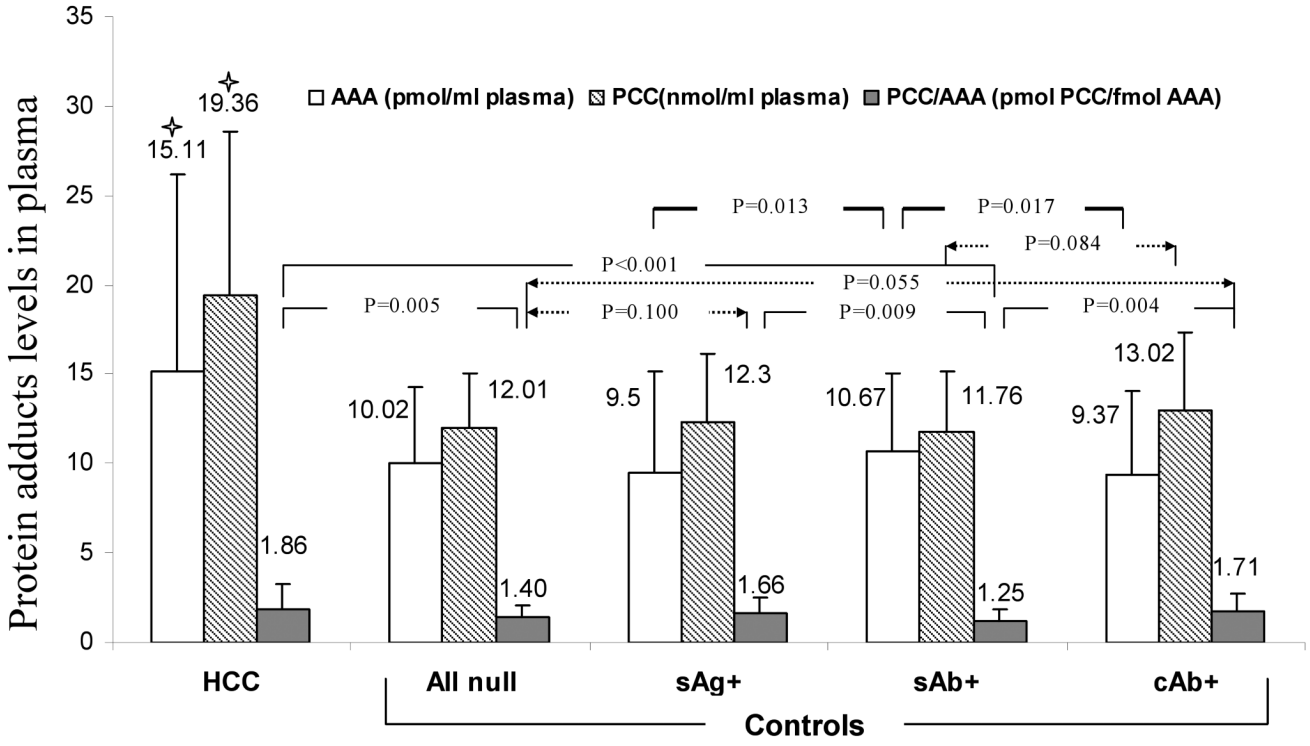

Figure 2. Comparison of Protein Adducts Levels among age and gender matched HCC and HBV sero-status groups (n=71 per group)

★ HCC patients had significantly higher levels of AAA and PCC than any HBV group of controls (P<0.001). Only p values less than 0.1 are indicated. Null (null for all HBV markers), sAg(+) (HBsAg(+) regardless of sAb or cAb status), sAb(+) (sAg(-)sAb(+)cAb(-)), and cAb (+) (sAg(-)cAb(+)).

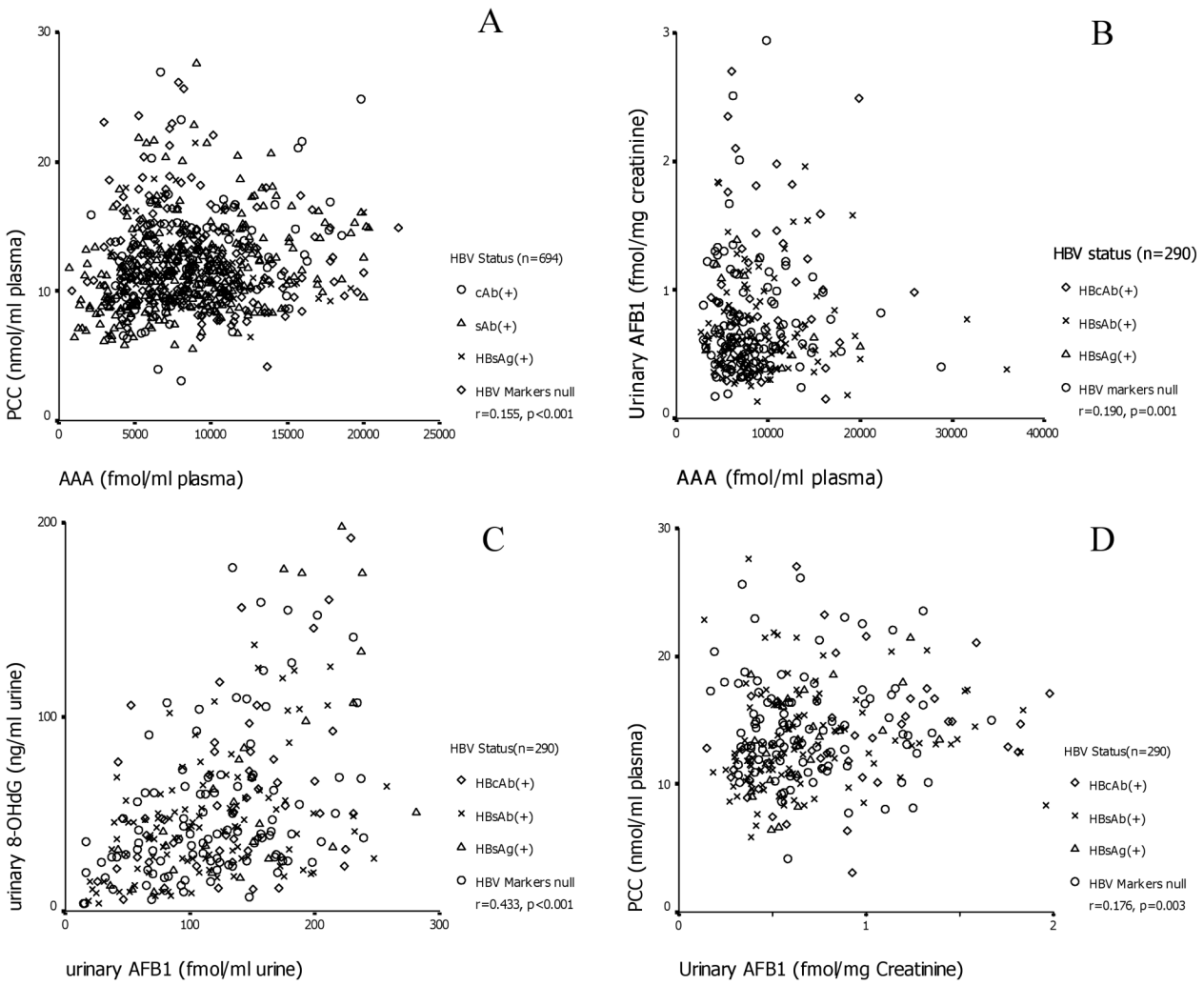

Figure 3.
Associations between plasma AAA and PCC (A), plasma AAA and urinary AFB1 (B), urinary AFB1 and 8-OHdG (C), urinary AFB1 and plasma PCC (D).

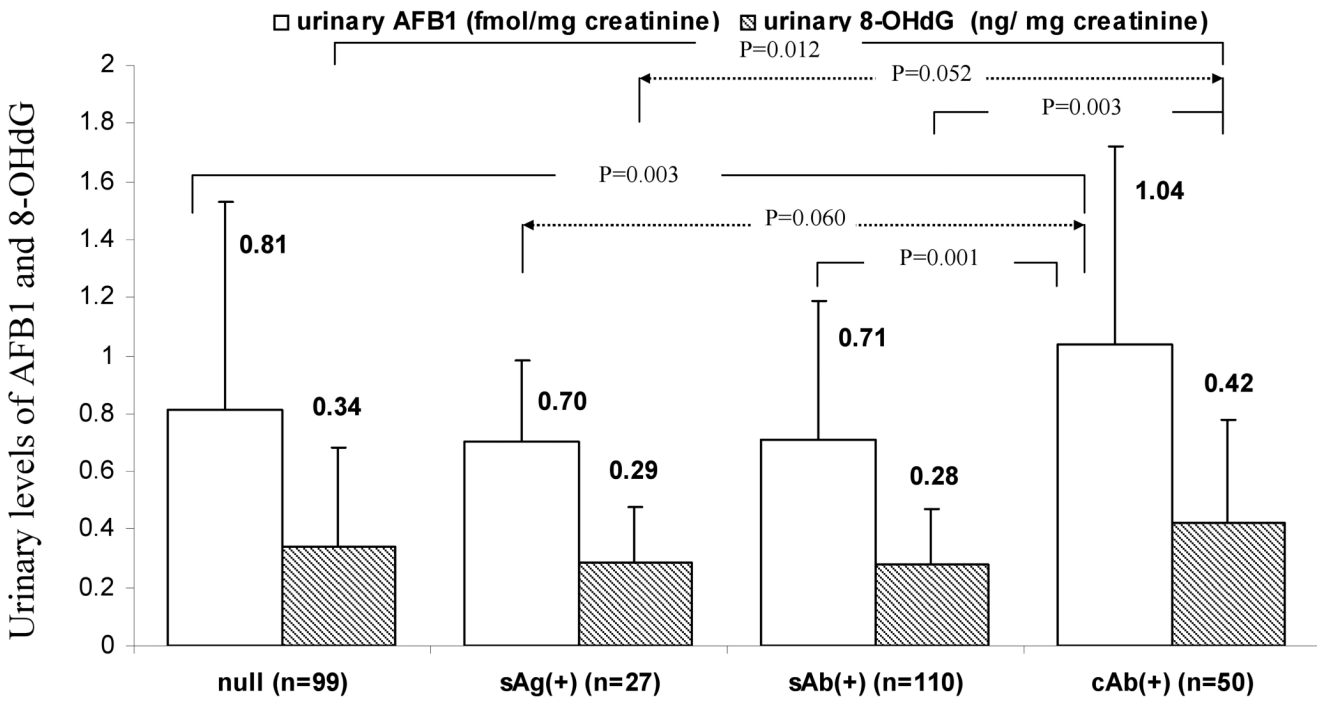

Figure 4. Comparison of Urinary AFB1 and 8-OHdG levels among HBV Sero-status groups in 290 adults from Nanning, China

Only p values less than 0.1 are indicated. Null (null for all HBV markers), sAg(+) (HBsAg(+) regardless of sAb or cAb status), sAb(+) (sAg(-)sAb(+)cAb(-)), and cAb(+) (sAg(-)cAb(+)).

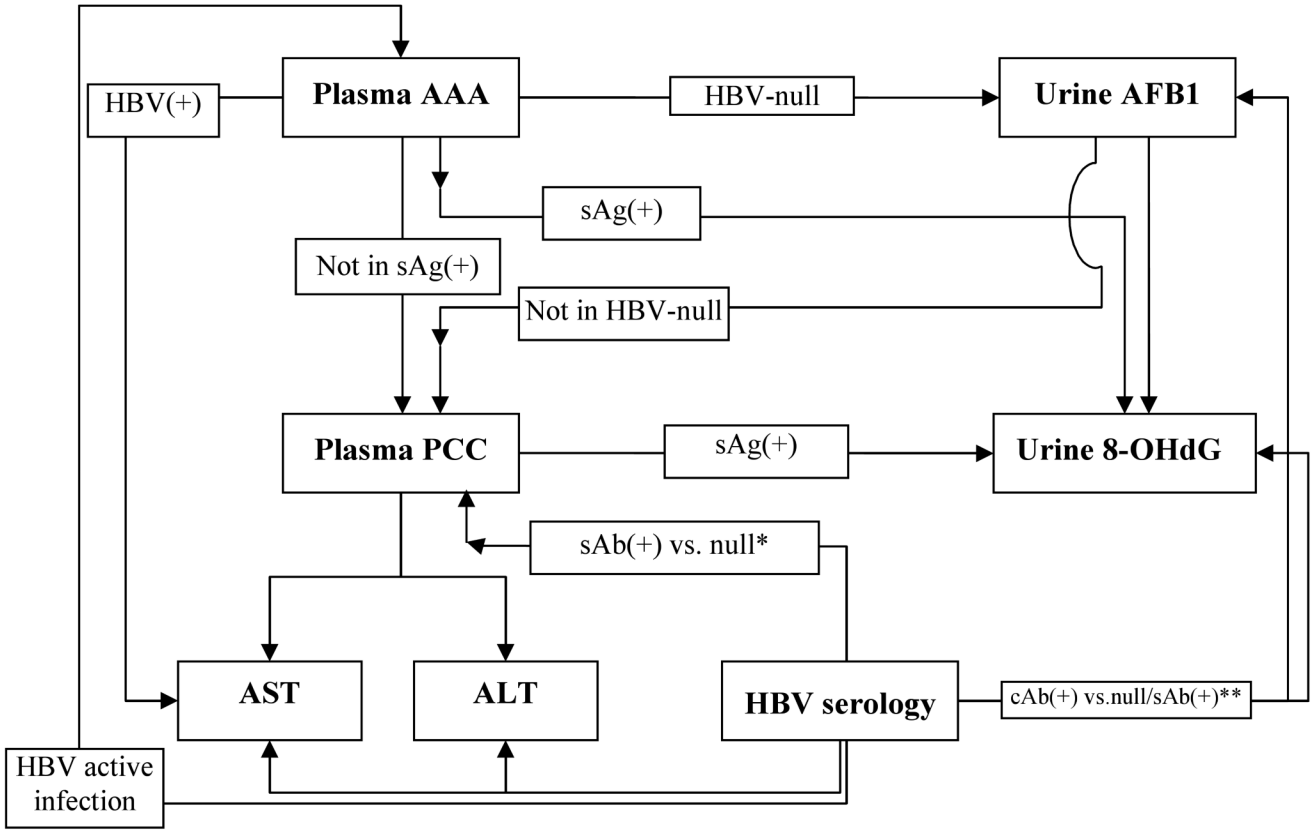

Figure 5.

Diagram of the association profile among biomarkers for oxidative stress, HBV infection and AFB1 exposure. Only significant associations are indicated. HBV status is specified when the associations are significant in sub-set(s) of HBV serology. Null (null for all HBV markers), sAg(+) (HBsAg(+) regardless of sAb or cAb status), sAb(+) (sAg(-)sAb(+)cAb(-)), and cAb (+) (sAg(-)cAb(+)). * Also significant between eAb(+) and eAb(-) among 87 sAg(-)cAb(+) subjects. ** Also significant between non-active HBV infection and HBV-free subjects.