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HBsAg, aflatoxins and primary hepatocellular carcinoma

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Summary

Nigeria is a very high risk area for primary hepatocellular carcinoma and this is the first study to utilize measurements of both hepatitis B virus status and aflatoxin levels in the same patients to determine the role of these factors in the causation of liver cancer in this environment.

We have shown that there is a higher prevalence of hepatitis B surface antigen ($P < 0.005$) and higher 'pathologic' serum levels of aflatoxins ($P < 0.05$) in patients with primary hepatocellular carcinoma than in matched controls.

It is considered that the results of this study may strengthen the hypothesis that hepatitis B virus may be an important aetiological factor in the development of primary hepatocellular carcinoma. Further work is in progress to correlate the level of aflatoxin serum albumin adducts with liver damage in order to assess the value of the albumin adduct as a marker of risk of liver cancer development.

Résumé

Nigeria est une trés haut risque aire pour le primaire carcinomé de foie cellules et ce ci la première étude à utiliser mesure de les deux niveaux de virus hépatite B statut et aflatoxines dans les même malades en determiné le rôle de ces facteurs en cancer de la foie de cet environnement.

Nous avons manifesté que it ya un plus haut fréquence antigene surface de hépatite B ($P < 0.005$) et plus haut niveaux pathologique de aflatoxines ($P < 0.05$) en malades avec primaire carcinome de toie cellules que dans les contrôles.

On a considéré que les résultats de cette étude avait augmenter, la hypothése que peut-etre virus hepatite B sont les facteurs important aetiologique a la developpement de primaire carcinome de foie cellules.

Ultereiux traveaux est en progrès pour correspondre le niveau de la composé l'aflatoxin sérum albumine avec les dommage foie - acide dés oxifribonucléique (DNA), afin d'acés la valeur de composé l'albumine comme la matiere de risque le développement de cancer de foie.

Introduction

Primary hepatocellular carcinoma (PHC) is a rare cancer in North America and Western Europe. On the other hand, it is one of the most common cancers in many parts of Africa and Asia where most of the world's population resides[1]. In Nigeria, it is the most common cancer in males and the fourth most common malignancy in both sexes[2]. Hepatitis B virus (HBV) has been implicated as a causative agent[3,4] but it has been observed that aflatoxins (AFs) may have a role in the aetiology of the PHC[5,6]. We have therefore examined the status of HBV and AFs in the serum of individual patient with PHC and compared this with a matched control at the University College Hospital, Ibadan, Nigeria. This is the first of such a study from this environment.

Materials and method

Twenty-two patients with PHC were studied between January and December, 1988. The diagnosis of PHC was based on histopathology of liver tissue obtained at needle-biopsy in all patients. For each patient

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diagnosed, a control who was matched to the case on sex and age (± 5 years) was sought in the Gastroenterology Ward of the hospital. These controls had acid peptic disease unrelated to liver diseases (as assessed by physical examination, liver function tests and abdominal ultrasonography) and were included in this study. Blood samples were collected from the subjects after being on only hospital diet for a week. The blood sample from the control was taken within two weeks of the index case. Blood samples were also taken from apparently healthy British caucasians who had not travelled to the tropics or sub-tropics in the six months before the venepuncture. All the samples were centrifuged, frozen at -20°C, transported to Liverpool School of Tropical Medicine and refrozen at -20°C. The sera were analysed for hepatitis B surface antigen (HBsAg) by ELISA test (Wellcozyne HbsAg, Bartford, U.K.). Also they were used for AF analysis using HPLC fluorescence detection after hexane partition and chloroform extraction[7]. This methodology permits detection of seven major AFs: B_1, B_2, M_1, M_2, G_1, G_2 and aflatoxicol and its sensitivity and reproducibility has been validated[8].

Statistical analysis was performed using the standard chi-square test 2 x 2 contingency tables.

Results

Twenty patients with PHC were males with a mean age of 48.3 years. The male controls had a mean age of 48.8 years. The mean age of the 2 female PHC patients was 45.3 years and their corresponding controls had a mean age of 43.5 years. The British caucasians were 20 males (mean age 48 years) and 2 females (mean age 35 years).

Sixteen (72.7%) cases with PHC were HBsAg positive compared with 8 (36%) controls ($P < 0.005$).

The serum AF levels were less than 17 pg/ml in the British caucasians; therefore we considered an AF level greater than 17 pg/ml as 'pathologic'. 'Pathologic' levels of AFs were consequently detected in 5 (22.7%) of the patients with PHC and in one (4.5%) of the controls ($P < 0.05$). Of the 5 PHC cases, 2 were HBsAg negative.

The types and amounts of AFs detected in the sera of the subjects are shown in Table 1.

Table 1: The types and amounts of serum 'pathologic' levels of aflatoxins in patients with liver cancer and their matched controls.

	Patient No.	Serum Aflatoxins (pg/ml)			
		AFM_1	AFG_1	AFL	AFB_2
Patients	1	—	1319	84	—
	4	14804	—	—	—
	8	13244	—	—	—
	11	—	9345	—	—
	12	—	—	172	—
Control	12	—	—	—	36

Discussion

The incidence of HBsAg positivity in the sera of the control group in this study (36%) is appreciably lower than the figure of 47% obtained in an earlier paper among the urban dwellers of Ibadan in a comparative study with the incidence among the rural dwellers of Igbo-Ora[9]. This observed difference within the same population may be a reflection of sampling variability. PHC, regarded as one of the world's commonest cancers[10] has a very high incidence in parts of tropical Africa (in particular Nigeria[2]) and Asia[11] and there is strong evidence for an aetiological association between HBV infections and PHC. It is based on sero-epidemiological studies, both retrospective[12] and prospective[12] and on the demonstration of HBV DNA integration with the host cell DNA in liver tumours and in liver cells of persistent carriers of HBV[14]. The results of the current study clearly show that in Nigeria, PHC is strongly associated with infection by HBV as assayed by seropositivity to HBsAg. Although all the PHC cases had probably been exposed to HBV, only about three-quarters were positive for HBsAg. That suggests that a proportion of the PHC cases could not be explained on the basis of HBV infection and other aetiological factors such as serum AF may be involved as primary carcinogens, co-carcinogens or promotive factors.

The considerable evidence implicating AF as a carcinogen in animals and the correlation studies demonstrating geographic concordance between populations with high AF exposure rates and a high incidence of PHC suggest that AF may be an important human carcinogen[15]. This view is supported by the earlier observations of Bababunmi and colleagues[16] who reported increased levels of AF in the urine of Nigerians with PHC. Our study has also shown that more patients with PHC have

serum 'pathologic' levels of AF than their matched controls. This variation is not likely to be due to greater exposure to AF by the PHC patients as they were on the same hospital diet for a similar period of time as the controls and were actually more anorexic due to the grave nature of their disease. It is important to note that AF-induced hepatocarcinogenesis is thought to stem from dose-linear formation by an epoxide metabolite[17]. It is also imporant to note that at present, it is not clear what levels of AFs in serum are carcinogenic. It may be that blood levels considered non-pathologic (less than 17 pg/ml) may contain toxic levels that are within the carcinogenic range when subjected to an appropriate induction period. Further work is in progress to correlate the level of AF-serum albumin adducts with liver damage. This is particularly relevant in assessing the value of the albumin adduct as a marker of the risk of developing liver cancer.

Clearly a larger study is desirable to confirm our findings.

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