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HBsAg and aflatoxins in sera of rural (Igbo-Ora) and urban (Ibadan) populations in Nigeria

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Summary

The purpose of this study was to screen for the presence of hepatitis B surface antigen and aflatoxins in the sera of 100 non-hospitalized individuals from the rural population of Igbo-Ora and 89 non-hospitalized individuals from the urban population of Ibadan, Nigeria. Hitherto, such a study as this has not been undertaken in this environment. The proportions of hepatitis B surface antigen carriage and serum 'pathologic' levels of aflatoxins were high (47 - 49%, 8.2 - 9.0% respectively) but varied very little between the two different populations sampled. These findings indicate that determined efforts should be instituted to reduce or eliminate hepatitis B virus infection and aflatoxin contamination of high risk foodstuffs from this environment.

Résumé

Le dessein de cette étude était l' écran pour la presence de l'antigene surface de hépatite B et aflatoxines dans 100 de la population rurale en Igbo-Ora qui ne sont pas dans l' hopital (non-hopitalise) et 89 de la population urban en Ibadan qui ne sont pas dans l' hopital en Nigeria. Jusqu 'ici, nous n' avons fait cette étude dans cet environment. Les proportions démarche de l' antigene surface de hépatite B et le niveau pathologique des aflatoxines étaient hauts (47 - 49%, 8.2 - 9.0% respectivement) mais varié un peu dans les differentes populations. Cette decouverte indique que les efforts étaient faire pour réduire ou bien éliminer les contagions avec virus hépatite B et contamination des nourritures (plus hautes risques) avec aflatoxines dans cet environment.

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Introduction

Primary hepatocellular carcinoma (PHC) is common in tropical countries such as Nigeria[1,2] where it is the commonest solid tumour found in males. Various factors including smoking, alcohol, parasites and sex hormones have been suggested as aetiologically important, but the major factors seem to be hepatitis B virus (HBV) and aflatoxin (AF)[3].

AFs, carcinogenic and toxic secondary metabolites of certain fungi, Aspergillus flavus and A. parasiticus are of considerable interest because of their presence in local foodstuffs in Nigeria[4,5]. Previous reports from this country have detected AFs in the blood of health blood donors[6,7] and of newly-admitted liver cancer patients[8]. However, in none of these studies was the HBV status of the general population in such studies indicated.

The present study was undertaken to screen for both HBV markers and AFs in the sera of rural and urban populations in Nigeria.

Method

One hundred and eighty-nine non-hospitalized Nigerians were studied. Of these, 100 were born and brought up or had lived continuously for ten years before commencement of this study in rural Igbo-Ora (I'Ora), Oyo State. These subjects are referred to in this analysis as rural population. The remaining subjects were born and reared or had lived

continuously for ten years in Ibadan, the capital city of Oyo State, prior to the commencement of the study. These subjects are referred to as urban populations. None of the subjects had had previous history of jaundice and they were all found on general physical examination to be in satisfactory general condition. The ages of the subjects were (0 to 2 were included), 5 ± 2, 10 ± 2, 25 ± 2 and 50 ± 2 years representing the age-structure of the society, the younger ones being proportionally more than the older ones.

Having been informed about the usefulness of the study, informed consents were obtained from the subjects or parents/guardians before enlisting each subject in to the study.

Blood samples were collected in March (environmental temperature = 21 - 36°C, dry). Thirty-one samples to determine the 'pathologic' threshold of serum AF were also taken from apparently healthy British caucasians who had not travelled to the tropics or subtropics in the six months before the venepuncture. They were 20 men and 11 women (mean age 40 ± 12 years). All the blood samples were centrifuged, frozen at -20°C until analysed. The sera were later analysed for hepatitis B surface antigen (HBsAg) by ELISA test (Wellcozyme HBsAg, Dartford, U.K.) and HBV DNA by spot hybridization[9]. Also they were used for AF analysis using HPLC fluorescence detection after hexane partition and chloroform extraction[10]. 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[11].

Differences between rural and urban populations were analysed statistically using the x^2 test.

Results

Forty-nine of 100 (49%) rural subjects and 42 of 89 (47%) urban subjects were consistently and reproducibly seropositive for HBsAg ($p > 0.05$). Two of the former and 5 of the latter were positive for both HBV DNA and HBsAg. When results were stratified for age, the HBV profile was not statistically significant in both groups ($p > 0.05$).

The total AF levels were less than 17 pg/ml in the British controls; therefore, we considered an AF level greater than 17 pg/ml as 'pathologic'. Consequently, 'pathologic' serum levels of AFs were detected in 8 (8.2%) of 97 tested rural subjects and in 7 (9.0%) opf 78 urban subjects ($p > 0.05$). When the various age groups were compared, there was no statistical difference between the urban and rural subjects. The types and amounts of AFs in the subjects are shown in the Table. There were so few subjects with the various types of AFs and the amounts of the AFs were so widely dispersed that it was not possible to draw any conclusions about differences in types and amounts of AF between rural and urban populations.

Table 1: The types and amounts of Aflatoxins detected in rural (Igbo-Ora) and urban (Ibadan) populations in Nigeria.

Age Group (Years)	Types and amounts (pg/ml) of Aflatoxins per Subject					
	AFB_1	AFB_2	AFM_1	AFM_2	AFG_1	AFG_2
Rural						
0 (up to 2)	—	—	156	—	2683	—
5 ± 2	—	—	20	—	—	—
10 ± 2	—	—	4984	—	6436	20
25 ± 2	—	—	—	24	—	—
50 ± 2	—	—	136	—	—	—
Urban						
0 (up to 2)	—	—	—	—	—	—
5 ± 2	—	—	—	24076	—	—
10 ± 2	2676	—	—	—	8828	—
25 ± 2	6532	—	—	—	—	—
50 ± 2	—	36	1272	—	—	—

Six (6%) of the rural subjects and 4 (4.5%) of the urban subjects had both 'pathologic' levels of AFs and HBsAg present in their sera.

Discussion

The prevalence of HBV infection in Nigerians is high compared to that described in other high-risk populations in Africa and Asia and, in fact, the rate of 47-49% HBsAg carriers in both populations is one of the highest ever recorded. A survey of blood donors in Senegal detected HBsAg in 14% of subjects under 50 years of age and 5% in donors over 50 years[12]. In adults used as subjects in case-control studies in Africa, the prevalence of HBsAg ranged from 2-3%[13] to 11-12%[14]. However, these figures are almost certainly underestimates, since they were obtained before the widespread use of ELISA methods. We have also shown that there was little difference in prevalence of HBsAg between the residents of rural when compared with urban areas suggesting that HBV transmission is common in both populations. The high prevalence of HBsAg in symptomless Nigerian populations in our study is of great interest since there now seems little doubt worldwide that the HBV is an important carcinogen with respect to PHC[15,16].

In either the rural or urban markets, peanuts, groundnut and oil palm products, rice, maize, yams (Diascorea spp), cassava (Mannihot spp) and dried fish constitute the staple foods for the majority of the inhabitants as the urban markets receive most of their foodstock from the rural areas. The populations are exposed to AFs by the consumption of these major food items that have been directly contaminated by the fungal strains during growth, harvest or storage. From the foregoing, it could be deduced that rural population has identical dietary exposure to AFs as their urban counterparts in Nigeria. It is therefore not suprising that a proportion of the residents regarded as healthy in the rural and urban areas have serum 'pathologic' levels of AFs and the levels varied very little in both populations. This is of great interest since although there is still much to be learned of the role of the AFs in the aetiology of liver cancer in man, impressive evidence of an association has been built from a number of field surveys]17]. However, at present, it is not clear what levels of AFs in serum are carcinogenic. It may be that blood levels considered 'non-pathologic' (below 17 pg/ml) may in fact be within the carcinogenic range when subjected to an appropriate induction period.

It seems reasonable to assume that the serum values of AFs may, in fact, be higher than at other times of the year as the present study was conducted at the peak of the dry season when the growth rate of the fungi which synthesize AFs is relatively slower[5].

AFs and HBV were frequently found to co-exist in the same individuals in this study. Though the precise mechanism of interaction is still unknown, available evidence suggests that HBV and AFs are the major factors aetiologically related to the development of liver cancer[3,18] and suggests that it is unwise to continue to ignore their importance in areas with a high incidence of liver cancer. In view of this, we plan to follow up our subjects who are both HBsAg positive and with high serum AF levels to assess their propensity for developing PHC. Meanwhile, efforts should be geared towards the prevention of HBV by vaccination programmes and prevention of AF contamination of high risk foodstuffs by adoption of better food storage and growth practices. It is however important to note that it was only one rural population and one urban population that were sampled and the finding made therefrom cannot be taken to be unequivocally representative of the pattern in all rural and urban populations of Nigeria.

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