Primary Liver Cancer Rate and Aflatoxin Intake in a High Cancer Area

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SUMMARY

Data have been accumulating in various parts of the world which suggest that there might be some relationship between the level of aflatoxin intake by certain populations and their primary liver cancer rate. The object of this work was to examine this relationship in the highest known liver cancer incidence area in the world, namely the Inhambane district of Mozambique. It was therefore necessary to obtain an estimate of the primary liver cancer rate for the total population which was more reliable than existing data, and to sample prepared meals throughout the district for aflatoxin analysis.

The age and sex distribution of the disease was obtained by means of a hospital registration programme and data on the absolute occurrence of the disease extracted from the health records of gold-miners originating from the study area. The two sets of data were used to calculate the specific cancer rates according to age and sex, in addition to the over-all cancer rate for the population. Values for the 1964 to 1968 and 1969 to 1971 periods were 35,5 and 25,4 per 100 000 per year, the disease occurring more than twice as commonly in males than in females.

The extent of aflatoxin contamination of prepared food consumed by the population was ascertained by means of the chemical assay of 880 meals collected at random. The mycotoxin was found in 9,3% of all samples, resulting in a mean contamination of all prepared food of 7,8 μg/kg wet food, and a mean daily per capita consumption of 222,4 ng/kg bodyweight (15 μg/adult/day). The pooling of these data with similar studies in lower incidence areas elsewhere revealed a significant correlation between the level of aflatoxin consumption and the liver cancer rate.

The results obtained represent both the highest primary liver cancer rate reported and the highest known aflatoxin intake.

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The anomalous distribution pattern of primary liver cancer (hepatocellular carcinoma) may be exploited in aetiological studies if the epidemiological situation is viewed as a natural experiment of immense proportion. The results are reflected in incidence statistics; only the pertinent variables leading to them must be unravelled. Undoubtedly this universal disease has a multifactorial aetiology, yet particularly in Africa south of the Sahara the distribution is patchy, and the incidence rate is frequently very much higher than that found in Europe or in the USA.[1] Such epidemic rates in confined geographical areas of one continent may well be due to one single additional agent in the environment, possibly arising from a set of circumstances involving natural resources, climatic and ethnic group factors.

Discovery of the exceedingly potent fungus-produced hepatocarcinogen, aflatoxin, has led to frequent conjectural associations with primary liver cancer.[2-4] Many other possible causes, e.g. siderosis, alcoholic beverages, tropical parasitism, malnutrition, plant alkaloids and virus infections have been considered, but systematic evaluation of all the evidence shows that the most credence may be assigned to the fungal toxin hypothesis.[5,6] There are nevertheless still objections to the hypothesis, and certainly some of the assumptions on which it was developed, such as the presence of a humid climate, have not withstood objective scrutiny.[7]

Many studies, usually based on the random sampling of stored staple foods without ensuring freedom from subjective bias, have demonstrated the presence of aflatoxin contamination in foods consumed by populations having a high primary liver cancer rate. Similarly, food eaten by ethnic groups relatively free of the disease, who live among the afflicted populations, is known in the experience of our laboratories to be virtually free of aflatoxin. Several other environmental factors may be associated with this flexible manner, and fallacious acceptance of the aflatoxin hypothesis could have disastrous consequences by unnecessarily depriving vast populations of a significant proportion of the national harvest, and subjecting them to troublesome control procedures.

Clearly, more precise evidence, obtained by means of diverse corroborative approaches, is required to test the aflatoxin hypothesis. Since chemical carcinogenesis is characteristically dose-related, the paramount epidemiological approach is to establish a dose-response to relationship. This can only be achieved by a costly programme of large-scale sampling of prepared food immediately before consumption, and a collateral cancer registration programme.

The highest recorded incidence of primary liver cancer is that for the Blacks of Mozambique.[8] Clinical observations by the joint Portuguese-South African team investigating the disease, suggested that the locality with the highest incidence rate was the Inhambane district, situated on the coast between Lourenço Marques and Beira, hence it was chosen as the study area. The unique frequency of the disease there has been confirmed indirectly by calculating the male adult rates from cases occurring in gold-miners recruited from extensive regions in Southern Africa.[9,10] The object of this study was to estimate average aflatoxin intake levels of the population in the area, and to obtain reasonably accurate primary liver cancer rate data. Aliquots of prepared meals were sampled immediately before consumption for the chemical assay of aflatoxin. The over-all cancer rate was estimated, using known data obtained from the health records of gold-miners originating from Inhambane, and equating these values with the age and sex distribution as found in our hospital registration programme.

LIVER CANCER INCIDENCE RATE

Ascertaining accurate cancer incidence data for the type of rural population usually studied, presents a task of enormous logistic proportions. A hospital incidence rate for our Mozambique study area of 16/100 000/year has been recorded.[11] Field investigations have, however, provided evidence that this value is an underestimate. Ideally, every member of the population should be under continuous medical surveillance for one year. This situation does in fact apply to that sample of the population which leaves the district to work on the gold mines in South Africa.

Thus over a period of 8 years, 208 574 man-years were spent by males originating from our study area on the mines, and while there, 185 developed primary liver cancer.[9,10] Concurrently, data on the age and sex distribution of 460 histologically confirmed cases were obtained at the main hospitals serving the area. Census data on the age and sex distribution of the local population were obtained from the 1970 census for the combined Sofala, Tete and Lourenço Marques districts, since those on Inhambane were not yet available. The age distribution of miners showed that three-quarters of them were aged between 20 - 39 years, and it was assumed that the miners' cancer rate represented this age group. Using the above data, it was possible to calculate over-all cancer rates from the known segment of the population. The statistical procedures used will be described in the final report on this study.

There appears to be a considerable decrease in the cancer rate over time in the study area.[10] The mine data were available for an 8-year period extending from 1964 to 1971. The first 5-year period and the last 3 years were calculated separately, and respective over-all rates of 35,5 and 25,4/100 000/year were found. The age-specific male and female rates in Table I are for the more recent 3-year period. Even this lower cancer rate of 25,4 for the total population is higher than any reasonably reliable rate for primary liver cancer reported elsewhere.

TABLE I. PRIMARY LIVER CANCER INCIDENCE RATES (10^5 p.a.) FOR INHAMBANE* AS CALCULATED FROM THE MEDICAL RECORDS OF MINERS, HOSPITAL REGISTRATION AND CENSUS DATA

Age group (yrs)	Males	Females	Total
0 - 9	1,3	0,3	0,8
10 - 19	13,8	9,2	11,7
20 - 39	71,2	30,8	50,2
40 - 49	76,8	36,4	55,9
50 - 59	107,3	27,1	63,8
60 - 69	65,9	17,7	41,3
70 -	30,3	—	15,4
Total	35,0	15,7	25,4

* Subdistricts of Zavala, Inharrime, Inhambane, Homoine, Massinga and Morrumbene.

AFLATOXIN INTAKE

Prepared food 'from the plate' was sampled systematically in the Inhambane District of Mozambique, and chemically assayed for total aflatoxins. Although 5 subdistricts within the Inhambane District are being studied, the results of only 880 meals are available to date, and therefore the values have been pooled to obtain a preliminary mean aflatoxin intake value for the whole region. Aflatoxin was found in 9,3% of the meals, resulting in a mean contamination of all prepared food of 7,8 μg/kg wet food. If a mean total daily food consumption of 2 kg/70-kg adult is assumed, the mean daily dose per person would be 222,4 ng/kg bodyweight. Thus on the average, adults consume 15,6 μg aflatoxin per day.

The significance of these high intake values in the highest known liver cancer area can best be assessed by comparison with similar studies in lower incidence areas, and by testing the dose-response relationship. Only two other similar studies, one in Thailand[12] and the other in Kenya,[13] have been completed. In Kenya, assuming that there were no cases of cancer in the age group 0 - 15 years, the study area was found to have an incidence of 3,2 cases/10^5/year and the aflatoxin intake was comparatively low (7,8 ng/kg/day). In Thailand the incidence rate was estimated to be more than double (7,3/10^5/year), and the intake was considerably higher (74 ng/kg/day). Some problems, however, were experienced in the assessment of cancer incidence in some areas,[14] but within both countries strong supportive evidence for a dose-response relationship was found when each study area was divided into high, medium and low incidence areas.

In Table II an attempt to standardise the available international data has been made. Cancer rates are those for the total population, and the aflatoxin intake values ignore the relatively small amounts of aflatoxin found in beer and possible trace amounts in food, which are difficult to measure. The pooled data show that over a wide range the cancer incidence appears to be linearly related to the logarithm of the level of aflatoxin intake (Fig. 1).

The apparently decreasing trend in Mozambique cancer rates[10] raises the anomaly of current exposure and intake values. As mentioned above, incidence rates that have been

TABLE II. SUMMARISED RESULTS OF STUDIES MEASURING PRIMARY LIVER CANCER INCIDENCE RATE AND AFLATOXIN INTAKE

Locality	Cancer rate (10^5/year)	Aflatoxin intake (ng/kg bodyweight/day)
Kenya — high altitude	**0,7**	**3,5**
Thailand — Songkhla	**2,0**	**5,0**
Kenya — middle altitude	**2,9**	**5,8**
Kenya — low altitude	**4,2**	**10,0**
Thailand — Ratburi	**6,0**	**45,0**
Mozambique — Inhambane	**25,4**	**222,4**

$y = 12{,}203 \log x - 7{,}384$
where y = cancer rate (10^5/year) and
x = aflatoxin intake.
$r = 0{,}918$ ($P<0{,}01$).

obtained for our study area range between 16 and 35. Within this range, with decreasing cancer rate, the correlation coefficient increases from 0,895 ($P<0{,}02$) to 0,959 ($P<0{,}01$) (Fig. 1). This finding is in accord with a predicted further decrease of the cancer rate, or alternatively may to some extent be due to unusually efficient cancer registration by means of using the records of gold-miners rather than local hospital registrations.

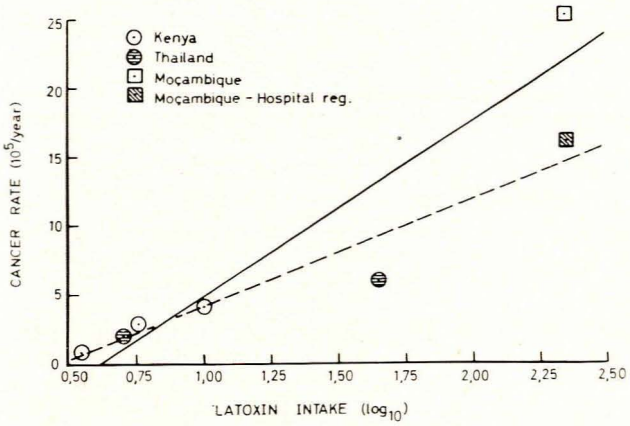

Fig. 1. Relationship between the level of aflatoxin intake of populations and the primary liver cancer rate, obtained by pooling available international data. Solid line: **The cancer rate for Mozambique was calculated by equating the incidence rate of gold-miners to that of the general population ($y = 12{,}203\ z - 7{,}384$; $P<0{,}02$).** Broken line: **Hospital registrations were used to calculate the cancer rate for Mozambique ($y = 7{,}63502 - 3{,}6162$; $P<0{,}01$).**

It was also deemed of interest to compare the Mozambique data with the regression line found in the Kenya study, which was based on adult cancer rates and independent intake values for males and females. For this purpose the proportional higher intake found for males in Kenya was assumed to apply to Mozambique males. The adult cancer rate for our study area was also calculated for each of the sexes as well as the 99% confidence limits of the Kenya regression line. The co-ordinates of our Mozambique data were found to be within the 99% confidence limits. This finding largely refutes objections to the small range covered by the Kenya data.

DISCUSSION

Similar studies, in particular the one performed in Kenya, yielded good evidence of a distinct association between the intake of aflatoxin by humans and subsequent development of primary liver cancer. The limitation of this study was that the cancer rates in the area examined were exceedingly low. The validity of such results is dependent on efficient cancer registration, since if only a few cases are missed in the lower incidence area, the dose-response relationship may be upset. The results of our work conducted in Mozambique are at the other extreme of the scale, and are shown to be quite compatible with the findings in lower incidence areas.

The anomaly of attempting to relate current cancer to current exposure ideally necessitates the use of a stable rural population. Although the Inhambane district remains relatively traditional, there are certainly signs of westernisation which inevitably affect dietary habits. Current data in Southern Africa suggest that westernisation is accompanied by a parallel decrease in the incidence of primary liver cancer, which implies that the disease appears to be potentially preventable. Such factors may be responsible for the observed decrease in the incidence rate in Mozambique, and it is noteworthy that our data are actually more compatible if the predicted decrease occurs.

The induction period of primary liver cancer may be shorter than previously believed, since recent evidence obtained from migrant workers from rural areas to the South African gold mines shows that there is a tendency for seasonal clustering of cases, a decrease in incidence after a period on the mines, and a very short tumour cell doubling time.[15]

Naturally, the association between cancer rate and toxin intake does not necessarily mean that the one is the cause and the other the effect. For example, aflatoxin levels may merely be an indicator of general carcinogenic mycotoxin contamination of foods. A systematic search for the presence of compounds other than aflatoxin, which are similar, has commenced in our laboratory. The action of the carcinogen may also be dependent on a condition of susceptibility of the liver, such as repeated regenerative cell cycles occurring after chronic liver damage from agents such as malaria and hepatitis virus.

Mean aflatoxin ingestion values must also be shown to be compatible with the biological facts. Experimentally, the acute, subacute and chronic pathological effects may be determined on appropriate animals, and if man is equally susceptible, these phenomena should be apparent in the population studied. Biochemically, metabolic characteristics associated with carcinogenicity can be identified by means of interspecies comparisons and some of these may be identified in man by means of studying available tissues and excretions. The plausibility of the hypothesis will ultimately depend on the degree of compatibility of the various parameters measured, particularly with reference to exceedingly high and low incidence areas.

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