Alpha-1-antitrypsin deficiency and hepatocellular carcinoma

Determination of Pi phenotypes using iso-electric focusing

J. VERGALLA, E. A. JONES, M. C. KEW

Summary

Alpha-1-antitrypsin (AAT) deficiency resulting from the homozygous protease inhibitor (Pi) ZZ and the heterozygous Pi Z states has been reported to be aetiologically associated with hepatocellular carcinoma (HCC). We studied the phenotype distribution of AAT variants (Pi) by iso-electric focusing on polyacrylamide gel and measured serum AAT concentrations by rocket immunoelectrophoresis in 80 unselected southern African Black patients with HCC and 103 age-, sex- and tribally matched control subjects. Aberrant (non-MM) phenotypes were present in 7 HCC patients (8.7%) and 13 controls (12.6%), an insignificant difference. None of the patients or controls had the Pi ZZ phenotype, while 4 HCC patients (5.0%) and 2 controls (1.9%) ($P > 0.05$) were found to be heterozygous carriers of the Z gene. No HCC patient had a subnormal serum AAT value, and the values in the patients were not lower than those in the controls. We conclude that AAT deficiency does not play an aetiological role in HCC in southern African Blacks. The 4 patients with the heterozygous Z phenotype did not have fibrolamellar carcinomas.

Some years ago we published the results of a study which showed that alpha-1-antitrypsin (AAT) deficiency did not play a significant role in the aetiology of hepatocellular carcinoma (HCC) in southern African Blacks.1 The same conclusion has since been reached in Greek,2 North American3 and English4 patients with this tumour. However, in a study in Denmark periodic acid-Schiff (PAS)-positive, diastase-resistant globules, thought to consist of asialo-AAT and to be indicative of AAT deficiency, were found in 12 of 69 patients with HCC.5 Moreover, non-MM protease inhibitor (Pi) phenotypes were reported to be more common in Italian patients with this tumour than in those with chronic liver disease.6 Our study included measurement of serum AAT concentrations, examination of non-tumorous liver tissue and tumour tissue for PAS-positive, diastase-resistant globules, and determination of Pi phenotypes by starch-gel electrophoresis.7 Since neither crossed electrophoresis nor iso-electric focusing was performed, it could be argued that heterozygous Pi Z phenotypes could have been missed. We therefore determined Pi phenotypes using iso-electric focusing on polyacrylamide gel, and also measured serum concentrations of AAT in a further series of HCC patients and appropriate controls.

Govindarajan et al.3 have recently reported that in a series of 124 patients with HCC the 3 individuals found to have the Pi MZ phenotype all had a relatively rare type of tumour known as fibrolamellar carcinoma,1 and the second purpose of the present investigation was therefore to establish whether these lesions were present in Black HCC patients with the Pi MZ phenotype.

Patients and methods

Eighty unselected Black adult male patients with histologically proven HCC were studied. There was no overlap between these patients and those previously reported,1 and their ages ranged from 19 to 74 years, with a mean of 43.6 years. Blood was taken at the time of diagnosis and before the onset of cytotoxic therapy. The controls were 103 apparently healthy Black males matched with the HCC patients for age and tribe. AAT concentrations were measured by the rocket immunoelectrophoresis method described by Weeke,7 using serum diluted 1:30 and a 1% agarose gel containing 10 lambda of monospecific antiserum to AAT per millilitre. Standards containing 4-30 mg AAT per millilitre were prepared from a standard plasma (Meloy; Springfield, Va., USA). Gels were electrophoresed by applying 200 V for 4 hours at 0°C.

Pi phenotyping was carried out according to the method of Kueppers8 except that preformed acrylamide gels, pH 4-5 (LKB; Bromma, Sweden), were used. Gels were electrophoresed by applying 30 W and 1400 V for 3 hours at 10°C, and were then fixed and stained according to LKB bulletin No. 1804. Standard sera of known Pi type and test sera were electrophoresed in parallel.

Results

Seven of the 80 patients (8.7%) with HCC and 13 of the 103 controls (12.6%) had non-MM Pi phenotypes; the difference was not significant (Table I). None of the patients or controls had the Pi ZZ phenotype. Four HCC patients (5.0%) and 2 controls (1.9%) ($P > 0.05$) were found to be heterozygous carriers of the Z gene. The serum AAT concentrations in the HCC patients were not lower than those in the control subjects (Table I). The lowest value in the HCC patients was 162 g/l and that in the controls 150 g/l. As in our previous study,1 serum AAT levels in the HCC

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patients were frequently found to be higher than the control values.

In turn, the AAT concentrations in the control subjects with different Pi phenotypes were higher than those encountered in healthy North American subjects. The mean serum concentrations in our laboratory in Washington are as follows: MM and MS 250 g/l, MZ 140 g/l, SZ 60 g/l and ZZ 50 g/l (J. Vergalla and E. A. Jones — unpublished data). In none of the 4 patients with the Pi MZ phenotype did histological examination of the tumour show a fibrolamellar carcinoma.

**Discussion**

Our failure to find either biochemical evidence of AAT deficiency or an increased number of homozygous or heterozygous Pi Z phenotypes on iso-electric focusing in polyacrylamide gel in Black patients with HCC confirms the findings of an earlier study. Further support for the conclusion that AAT deficiency does not play a role in the causation of HCC in southern African Blacks is provided by the recently published study of Cohen et al. Using a specific immunoperoxidase technique in addition to PAS staining with and without predigestion with diastase, Reintoft and Hagerstrand detected globules with AAT deficiency. To be certain that these globules consist of asialo-AAT it is necessary to use a specific staining technique such as immunoperoxidase or immunofluorescence, neither of which was used in our earlier study.

Using appropriate methods, AAT deficiency has not been shown to be a significant causal factor in HCC in Greek, North American, West African or English patients. Fargion et al. found normal serum AAT levels but an increased prevalence of non-MM Pi phenotypes in Italian patients with HCC. However, they used the same method of Pi phenotyping as was used in our earlier study, and they did not examine tissue for globules of asialo-AAT. The earlier 'positive' studies of Berg and Eriksson and Norkin and Campagna Pinto were based solely on the finding of PAS-positive, diastase-resistant globules. Using immunoperoxidase and PAS staining with and without predigestion with diastase, Reintoft and Hagerstrand detected globules of asialo-AAT in tumour tissue from 12 of 69 patients, and in 8 of these the globules were also present in the non-tumorous liver. However, they did not measure serum AAT concentrations or determine the Pi phenotypes in their patients. AAT deficiency might therefore predispose to the development of HCC in some parts of the world. This tumour appears to be multifactorial in causation, and it is conceivable that different causal factors are operative in different parts of the world.

AAT is known to be an acute-phase reactant, and its serum concentration may increase considerably during infections. The presence of low-grade chronic parasitic infestations might explain the higher values obtained in the Black controls than in North American Whites.

In a series of 124 patients with HCC only 3 were found to have the Pi MZ phenotype and, in contrast to the others, these patients had the fibrolamellar variant of HCC, which occurs particularly in non-cirrhotic livers in young female patients and carries a relatively good prognosis. None of the 4 patients in the present study found to have the MZ phenotype had fibrolamellar carcinomas. Other analyses of AAT deficiency in HCC have not commented on the histological features of the tumours.

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**REFERENCES**


**TABLE I.** Pi PHENOTYPES AND SERUM AAT CONCENTRATIONS IN 80 PATIENTS WITH HCC AND 103 CONTROL SUBJECTS

<table>
<thead>
<tr>
<th>Pi phenotype*</th>
<th>Serum AAT concentration (g/l)</th>
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<tbody>
<tr>
<td></td>
<td>Mean</td>
</tr>
<tr>
<td><strong>HCC patients</strong></td>
<td></td>
</tr>
<tr>
<td>MM 73 (91,2)</td>
<td>520</td>
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<tr>
<td>MS 3 (3,7)</td>
<td>507</td>
</tr>
<tr>
<td>MZ 4 (5,0)</td>
<td>477</td>
</tr>
<tr>
<td><strong>Control subjects</strong></td>
<td></td>
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<tr>
<td>MZ 90 (87,4)</td>
<td>334</td>
</tr>
<tr>
<td>MS 10 (9,7)</td>
<td>279</td>
</tr>
<tr>
<td>MZ 1 (1,0)</td>
<td>219</td>
</tr>
<tr>
<td>SS 1 (1,0)</td>
<td>201</td>
</tr>
<tr>
<td>SZ 1 (1,0)</td>
<td>618</td>
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*Percentages are given in parentheses.