Alpha₁-antitrypsin levels and phenotypes and hepatitis B serology in liver cancer

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Summary Serum levels of alpha₁-antitrypsin (α_1 AT) were measured by radial immunodiffusion and phenotypes were determined by electrofocusing in acrylamide gel in 39 patients with hepatocellular carcinoma (HCC) positive for serum hepatitis B surface antigen (HBsAg), 41 patients with HCC negative for serum HBsAg, and 160 age- and sex-matched hospital controls. There was no difference between the control series and either of the two HCC groups with respect to α_1 AT phenotype pattern; also, there was no evidence of association between HCC and either the M₂ allele or any of the α_1 AT deficiency phenotypes. However, HCC cases negative for HBsAg had significantly higher serum α_1 AT values (mean 665 ± 26 mg 100 ml⁻¹) than HCC cases positive for HBsAg (mean 571 ± 23 mg 100 ml⁻¹), who in turn, had significantly higher α_1 AT values than hospital controls (mean 434 ± 13 mg 100 ml⁻¹). These results indicate that (i) in Greece, as in other high HCC incidence countries, genetically determined α_1 AT deficiency is not aetiologically important; (ii) the increase of serum α_1 AT is an important correlate of HCC with possible aetiological significance and diagnostic potential and (iii) HBsAg-positive HCC and HBsAg-negative HCC are manifest differently as well as being aetiologically distinct.

Alpha₁-antitrypsin (α_1 AT) is one of nine distinct plasma proteins which have been characterized as protease inhibitors (Harpel, 1983); it is a glycoprotein synthesized in the liver and it is responsible for about 90 per cent of the tryptic inhibitory capacity of human serum (Sharp, 1976; Kew *et al.*, 1978; Morse, 1978; Chio & Oon, 1979). α_1 AT is under genetic control, and more than 30 codominant alleles at a single chromosomal locus have been identified (Chapuis-Cellier, 1975; Cox, 1978; Morse, 1978). Some of these alleles (particularly the alleles Z, I, S, and perhaps P and other less common ones) have been associated with serum α_1 AT deficiency of varying degree (Chapuis-Cellier, 1975; Morse, 1978; Chapuis-Cellier & Arnaud, 1979) and with the presence in the liver of periodic acid-Schiff(PAS)-positive, diastase-resistant globules (Sharp, 1976; Morse, 1978; Palmer *et al.*, 1980). There appears to be an intriguing link between α_1 AT and hepatocellular carcinoma (HCC) but the evidence is not clear cut (Sharp, 1976; Kew *et al.*, 1978; Morse, 1978; Kelly *et al.*, 1979). We have studied the association between α_1 AT and HCC in a large group of HCC cases and matched controls, using adequate laboratory procedures "blindly" (with respect to disease status), in a European population with an unusually high incidence of HCC (Trichopoulos *et al.*, 1982).

Patients and methods

Two hundred and forty patients were studied; they were Caucasians, of Greek nationality and residence, hospitalized in one of eight large hospitals of Athens during a 15-month period in 1976 and 1977. Among them 80 had HCC (confirmed histologically in 47 cases and by diagnostic alpha-fetoprotein (α FP) values in the remaining 33 cases); for each of them two control patients, matched for age and sex, were selected from the same hospitals with diagnoses other than neoplasm or liver disease. Among HCC patients ("cases") 69 (86%) were males and the average age was 63 years; among comparison patients ("controls") the corresponding figures were 138 males (86%) and 62 years. All patients were interviewed, and blood samples were taken from each.

Hepatitis B serologic markers were determined in all of the sera by radioimmunoassay; samples of 39 cases (49%) and 12 controls (8%) were found to be positive for hepatitis B surface antigen (HBsAg) and were considered to have chronic infection. The two HCC groups (HBsAg positive and HBsAg negative) were of similar age and sex (mean age, 63 years in both series; proportion of males 85% and 88% respectively). Therefore, age and sex adjustments were not necessary for comparisons between controls and either or both of the two HCC groups (Trichopoulos *et al.*, 1978).

Serum levels and phenotypes of α_1 AT were determined by radial immunodiffusion and

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electrofocusing in acrylamide gel, respectively (Vesterberg, 1973; Chapuis-Cellier, 1975). All determinations were performed blindly in the Department of Clinical Biochemistry of the Hospital "Edouard Herriot" in Lyon, France.

Results

Table I shows the distributions of HCC cases and controls by HBsAg status (positive or negative) and α_1 AT phenotype pattern. The four distributions are very similar and the observed differences can easily be explained by chance. Thus, comparison of all HCC cases with all controls, with respect to the four most common α_1 AT phenotypes (and a fifth group combining all the "other" less common ones) gives a χ^2 with 4 degrees of freedom of 2.56, corresponding to $P > 0.5$. Furthermore, there is no evidence in the present data that phenotypes associated with α_1 AT deficiency are over-represented among HCC cases; on the contrary, two heterozygotes for the Z allele were included in the control series whereas none was found among the cases. Lastly, among the cases 6% were homozygous and 14% heterozygous for the M_2 allele; among controls the corresponding figures were almost identical (7% and 11%, respectively) providing no support to the hypothesis that this allele is associated with a substantial increase of HCC risk.

Table II shows mean values of serum α_1 AT (in mg 100 ml⁻¹) in HCC cases and controls by α_1 AT phenotype and, for HCC cases, by HBsAg status.

The mean value of serum α_1 AT is higher in HCC cases than in hospital controls (by ~40%) and the difference is highly significant ($P < 10^{-9}$). Although HCC cases of both subgroups have elevated α_1 AT values the elevation is more marked among HBsAg-negative cases than among HBsAg-positive cases; the corresponding mean values are ~50% and 30% higher than the mean value among controls and they differ significantly from each other ($P < 0.01$) as well as from the mean value of controls ($P < 10^{-9}$ and $P < 10^{-6}$ respectively). It is of interest that the pattern HCC (HBsAg-) > HCC (HBsAg+) > Controls is evident not only in the total but also within every single α_1 AT phenotype (with only one marginal exception, in M_1M_2). There was no significant (or even similar) difference in the average level of serum α_1 AT between HBsAg positive and negative individuals in the control group; the mean values were 489 and 430 mg 100 ml⁻¹ respectively, and the P value for a t -test of their difference is 0.30. Recent reports (Trichopoulos *et al.*, 1980; Lam *et al.*, 1982) indicated that tobacco smoking may cause HBsAg-negative HCC. Since tobacco smokers in general have elevated concentrations of serum α_1 AT, we have explored whether the very high values of α_1 AT in HCC (and particularly in HBsAg-negative HCC) could reflect a specific association between α_1 AT and tobacco-related HBsAg-negative HCC. There is some evidence in the present data that this may be so, but it is far from conclusive. Among patients with HBsAg-negative HCC, markedly elevated concentrations of serum α_1 AT (>600 mg 100 ml⁻¹) were found in 21/31 current smokers (68%) but only in 5/10 non-smokers (50%); however, the

Table I Distribution of HCC cases and controls by HBsAg status and α_1 AT phenotype

AAT phenotype	HCC cases			Controls		
	HBsAg(+)	HBsAg(-)	Total (%)	HBsAg(+)	HBsAg(-)	Total (%)
M_1M_1	20	18	38 (47)	5	80	85 (53)
M_1M_2	1	5	6 (8)	1	10	11 (7)
M_1M_3	13	11	24 (30)	5	29	34 (21)
M_2M_2	3	2	5 (6)		11	11 (7)
M_2M_3	1	3	4 (5)	1	5	6 (4)
M_3M_3			(0)		5	5 (3)
M_1I					2	2
M_1S					1	1
M_1F					1	1
M_1N_2	1		1			
M_2P					1	1
M_2S		1	1			
M_3P					1	1
M_3S		1	1			
M_3Z					2	2
Total	39	41	80 (100)	12	148	160 (100)

Table II Concentration of serum α_1 AT in HCC cases (positive and negative for HBsAg) and in controls, by phenotype. Mean values (and standard errors) in mg 100 ml⁻¹

α_1 AT phenotype	HCC cases				Total		Controls	
	HBsAg(+)		HBsAg(-)				Total	
	n	$\bar{x}$	n	$\bar{x}$	n	$\bar{x}$	n	$\bar{x}$
M ₁ M ₁	20	601	18	675	38	636	85	451
M ₁ M ₂	1	378	5	619	6	579	11	414
M ₁ M ₃	13	579	11	645	24	609	34	439
M ₂ M ₂	3	479	2	888	5	643	11	445
M ₂ M ₃	1	440	3	661	4	605	6	361
M ₃ M ₃							5	415
other ^a	1	466			1	466	3	300
other ^b			2	578	2	578	5	312
Total	39	571	41	665	80	619	160	434
(standard error)		(23)		(26)		(18)		(13)

^aPhenotypes not reported to be associated with α_1 AT deficiency (M₁F, M₁N₂, M₂P, M₃P).

^bPhenotypes reported to be associated with α_1 AT deficiency (M₁I, M₁S, M₂S, M₃S, M₃Z).

Table III Distribution of 41 HCC cases negative for HBsAg by reported smoking habits before the onset of the disease and serum level of α_1 AT (above or below 600 mg 100 ml⁻¹).

Serum α_1 AT	Non (and Ex) smokers	Smokers (cigarettes day ⁻¹)				Total ^b
		1-10	11-20	21-30	31+	
600+	5	1	11	4	5	26
-599	5 ^a	2	3	2	3	15
Total	10	3	14	6	8	41

^aOne of these patients may have been a regular light smoker (repeated interview).

^bfor trend (1 d.f.) = 0.5; $P > 0.25$.

difference is neither statistically significant nor dose-dependent (Table III).

We have also investigated whether, among HCC patients, there is a positive correlation between the serum concentrations of α FP (after log-transformation) and α_1 AT. There is no such evidence in the present data; among HBsAg-positive HCC patients the correlation coefficient is +0.04, whereas among HBsAg-negative HCC patients the corresponding value is +0.05.

Discussion

The association between α_1 AT and HCC has been studied from several points of view. Individuals who are homozygous for the Z allele (and perhaps other alleles associated with α_1 AT deficiency) have an increased risk for HCC, and many of them have the characteristic globular bodies in their livers. However, it is not clear whether individuals who

are heterozygous for these alleles are at increased risk for HCC; many studies have noted modest associations but several others have not, even though heterozygotes have consistently the same characteristic globules in their livers (studies reviewed by Sharp, 1976; Kew *et al.*, 1978; Morse, 1978; Kelly *et al.*, 1979; Sizaret *et al.*, 1981; Spech & Liehr, 1982). Kew *et al.* (1978) have summarized the evidence by stating that α_1 AT deficiency could be an occasional cause of the tumour in most parts of the world where HCC occurs sporadically, but it is not a numerically important cause in Africa and the Far East where HCC is common. Our findings support this conclusion and permit its generalization to other populations where HCC is common, besides those of Africa and Asia. On the other hand, we did not confirm the associations of HCC with the F or the M₂ alleles, which were reported by Theodoropoulos *et al.* (1976) and Sizaret *et al.* (1981), respectively. Since the present study is larger than the other two we are inclined to

believe that the earlier findings were, perhaps, fortuitous.

Elevated values of serum α_1 AT in HCC cases have been noted by Kew *et al.*, (1978), Chio & Oon (1979), and Matsuzaki *et al.* (1981). Our findings confirm the results of the earlier investigations and indicate that the elevation is sufficiently marked to be aetiologically intriguing and diagnostically useful. Furthermore, we have found that serum α_1 AT values are, on the average, higher in HBsAg-negative cases of HCC than in HBsAg-positive cases of this tumour—an observation not reported previously. Although the difference is not sufficiently large to be of clinical importance it suggests that the aetiologic heterogeneity of HCC is reflected not only in the epidemiologic parameters but also in the laboratory findings of the aetiologic subgroups.

The molecular, biochemical and histological aspects of the association between α_1 AT and HCC have been reviewed elsewhere (Sharp, 1976; Morse, 1978; Palmer *et al.*, 1980; Spech & Liehr, 1982). However, the differential increase of α_1 AT in HBsAg-positive and HBsAg-negative HCC, if real, calls for explanation. Several possibilities exist. Tobacco smoking has been found to increase the

serum levels of α_1 AT by ~20% (Lellouch *et al.*, 1979) and this proportional excess may also concern the tobacco related HCC cases. It should be noted, also, that cirrhosis is associated with reduced levels of serum α_1 AT (Chio & Oon, 1979; Matsuzaki *et al.*, 1981), and cirrhosis (in Greece, at least) is more frequently associated with HBsAg-positive than with HBsAg-negative HCC (Trichopoulos *et al.*, 1982).

Palmer *et al.* (1980), using ultrastructural, histochemical and immunocytochemical methods have demonstrated the occasional parallel emergence of α FP and α_1 AT as tumour tissue markers in malignant hepatoma (HCC). It is, therefore, surprising that a significant positive association was not evident between the serum values of α FP and α_1 AT in the present study or in the earlier study of Chio & Oon (1979). Although chance is a likely explanation for the absence of a significant association, it is also possible that the epigenetic emergence of α_1 AT as tumour tissue marker is not frequently accompanied by an increase in the serum levels of α_1 AT (Palmer *et al.*, 1980).

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