α_1-ANTITRYPSIN PHENOTYPE PiMZ, A RISK FACTOR FOR LIVER CIRRHOSIS BUT NOT FOR LIVER CANCERS IN THAILAND

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Abstract. The risk of developing liver cirrhosis, hepatoma (HCC) and bile duct carcinoma (BDC) have been associated with homozygous α_1-antitrypsin (AT) deficiency especially linked to the Z allele. While the association between liver cancers and AT deficiency remains debatable, the risk of adult AT deficiency carriers to develop liver cirrhosis has not been assessed quantitatively. Liver cancers and liver diseases with subsequent liver cirrhosis are highly prevalent in tropical countries such as Thailand and heterozygous AT phenotypes are rather common in this country as well. The aim of the study was to assess the risk of developing liver cirrhosis, HCC and BDC by means of case-control studies with Thai patients and controls in connection with AT deficiency. For hepatitis, HCC and BDC no association with AT deficiency was detected. Carriers of PiMZ phenotype in Thailand have a high risk to develop liver cirrhosis (odds-ratio of 10.8; 95% confidence interval = 1.3-88.1). Patients with predisposing diseases should be screened for Pi phenotypes so that rigorous measures to combat the occurrence of liver cirrhosis can be implemented.

INTRODUCTION

α_1-Antitrypsin (AT), also termed α_1-protease inhibitor, accounts for approximately 90% of the trypsin inhibiting capacity of human serum. AT is the principal inhibitor of leukocyte elastase, but is also active against other proteolytic enzymes such as chymotrypsin, plasmin, thrombin and bacterial protease (Tschesche *et al*, 1971). The synthesis of AT is determined by a pair of genes at the Pi locus. Isoelectric focusing was the method chosen previously to identify different phenotypes. The application of the more sophisticated method of restriction of fragment length and direct DNA sequence analysis increased the number of different alleles to move than 30 variants known presently (Brantly *et al*, 1988). Some of the genetic variants have low serum levels of AT. Pathological conditions associated with AT deficiency have been linked especially to the Z allele. Besides, early development of pulmonary emphysema in patients suffering from PiZZ phenotype, and increased risk for various liver diseases in connection with AT of the Z type has been investigated recently. Heterozygous AT deficiency is said to play a role in the development of hepatocellular carcinoma (HCC) (Craig *et al*, 1975; Sveger, 1976; Sharp, 1982; Carlson and Eriksson, 1985; Hutchison, 1988), bile duct carcinoma (BDC) (Rabinovitz *et al*, 1992) and hepatic cirrhosis in adults (Eriksson and Hägerstrand, 1974; Morin *et al*, 1975; Kueppers *et al*, 1976; Cox and Smyth, 1983). The association of BDC with AT deficiency remains uncertain. For HCC, the diagnosis of AT deficiency was based on the results of tissue staining for AT globules. The presence of the globules was assumed to indicate homozygous (ZZ) or heterozygous (MZ) AT deficiency. Very often in these cases, a true determination of the phenotypes of cancer patients was not done (Robinovitz *et al*, 1992). The association between hepatic cirrhosis in adults and AT deficiency had been described without a quantitative determination of risk.

In tropical countries, like Thailand, liver diseases such as hepatitis, cirrhosis, HCC and BDC are common. A wide variety of different AT phenotypes, at least for certain subgroups, have been found in Thailand (Pongpaew and Schelp, 1980). Considering the frequency of liver diseases and the common

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occurrence of heterozygous AT phenotypes in this country, the objective of this investigation was to conduct case-control studies, to quantitatively assess the risk of AT heterozygous phenotypes to develop important liver diseases.

MATERIALS AND METHODS

Ninety-six patients suffering from hepatitis, 57 from liver cirrhosis, 42 from HCC and 12 from BDC from 3 hospitals in Bangkok were selected as cases. One hundred and nineteen patients, admitted to the hospitals for various diseases not related in any way to the lung or liver, and 88 healthy blood donors were selected to serve as controls. All individuals selected for the study were Thai nationals of Asian origin. The cases and controls were age- and sex-matched. Matching by age was done within a range of ± 5 years. The age range of hepatitis patients and controls varied from 14 to 72 years; for cirrhosis from 33 to 75 years; HCC from 30 to 85 years, and BDC from 34 to 65 years. Most of the subjects studied were males. The per-centage of males in the cases and controls was 79.2% for hepatitis, 100% for cirrhosis, 92.9% for HCC and 91.7% for BDC. Consent to participate in this study was obtained from all patients and controls. The study protocol was approved by the human research committee of the Faculty of Tropical Medicine.

AT phenotypes were determined by thin layer isoelectric focusing (TLIEF) on polyacrylamide gel (Allen *et al*, 1974; Pongpaew and Schelp, 1980).

To test significant differences between the distribution of AT phenotypes the chi-square test was applied. For risk assessment the ordinary odds-ratio was calculated. For the statistical analysis the SPSS program on a PC was used.

RESULTS

The distributions of AT phenotypes in cases and controls are given in Table 1. Variation of phenotypes was rare in HCC patients and absent in BDC cases. A statistically significant difference in Pi variations between cases and controls was only observed for liver cirrhosis. An increased risk could only be established for liver cirrhosis when all heterozygous AT phenotypes against PiMM (Table 2) were considered. The odds-ratio increased when only PiMZ was taken into account (OR 10.76; CI = 1.31-88.1).

Table 1

AT phenotypes in liver disease patients and controls.

Group	AT phenotype						Total
	MM	MS	MZ	SS	FM	IM	
Hepatitis	91	1	4	0	0	0	96
Controls	91	2	1	1	0	1	96
Cirrhosis	46	1	9*	0	1	0	57
Controls	55	1	1*	0	0	0	57
HCC	41	0	1	0	0	0	42
Controls	39	2	0	0	0	1	42
BDC	12	0	0	0	0	0	12
Controls	12	0	0	0	0	0	12
All cases	190	2	14*	0	1	0	207
Percent	91.79	0.97	6.76	0	0.48	0	100
All controls	197	5	2*	1	0	2	207
Percent	95.17	2.41	0.97	0.48	0	0.97	100

* Significant difference $p < 0.01$

DISCUSSION

In this investigation, the Pi phenotype frequency determined for the controls was similar to previous results obtained from a population survey done in Bangkok (Pongpaew and Schelp, 1980).

No association was found between heterozygous AT phenotypes, hepatitis and liver cancers under

Table 2

Odds-ratio of liver diseases in relation to heterozygous AT phenotypes.

Disease	Odds-ratio	95% CI
Hepatitis	1	0.28-3.57
Cirrhosis	6.58	1.39-31.19
HCC	0.32	0.03-3.17
BDC	0	0

investigations, *ie* HCC and BDC. As far as liver cancers are concerned, the result is in accordance with the findings of other investigators (Rabinovitz *et al*, 1992). Carriers of the phenotype PiMZ run a very high risk of developing liver cirrhosis.

A widely accepted theory, which attempts to explain the risk for the liver especially in individuals with the mutant Z allele, assumes that an accumulation of aggregated AT causes a necroinflammatory change in the liver cells, which is irreversible once the process is initiated (Carrell, 1986).

Patients with predisposing diseases known to develop into liver cirrhosis should be screened according to their Pi-types, to assess their value as predictive indices.

The risk of PiMZ carriers to develop liver cancers might be concealed by strong environmental factors which override the genetic factor. Thus, hepatitis and aflatoxin are widespread environmental risks in Thailand, as far as hepatoma is concerned. *Opisthorchis viverrini* is endemic in Thailand, has a high prevalence rate, and is associated with the occurrence of BDC (Flavell, 1981).

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REFERENCES

Allen R, Harley RA, Talamo RC. A new method for determination of alpha-1-antitrypsin phenotypes using isoelectric focusing on polyacrylamide gel slaps. *Am J Clin Pathol* 1974; 62 : 732-9.

Brantly M, Nukiwa T, Crystal RG. Molecular basis of alpha-1-antitrypsin deficiency. *Am J Med* 1988; 84 (suppl 6A) : 13-31.

Carlson J, Eriksson S. Chronic "cryptogenic" liver disease and malignant hepatoma in intermediate alpha-1-antitrypsin deficiency identified by a Pi Z-specific monoclonal antibody. *Scand J Gastroenterol* 1985; 20 : 835-42.

Carrell RW. Alpha-1-antitrypsin: molecular pathology, leukocytes and tissue damage. *J Clin Invest* 1986; 78 : 1427-31.

Cox DW, Smyth S. Risk for liver disease in adults with alpha-1-antitrypsin deficiency. *Am J Med* 1983; 74 : 221-7.

Craig JR, Dunn AEG, Peters RL. Cirrhosis associated with partial deficiency of alpha-1-antitrypsin: a clinical and autopsy study. *Hum Pathol* 1975; 6 : 113-20.

Eriksson S, Hägerstrand I. Cirrhosis and malignant hepatoma in alpha-1-antitrypsin deficiency. *Acta Med Scand* 1974; 195 : 451-8.

Flavell DJ. Liver-fluke infection as an aetiological factor in bileduct carcinoma in man. *Trans R Soc Trop Med Hyg* 1981; 75 : 814-24.

Hutchison DCS. Natural history of alpha-1-protease inhibitor deficiency. *Am J Med* 1988; 84 (suppl 6A): 3-12.

Kueppers F, Dickson ER, Summerskill WHJ. Alpha-1-antitrypsin phenotypes in chronic active liver disease and primary biliary cirrhosis. *Mayo Clin Proc* 1976; 51 : 286-8.

Morin T, Martin JP, Feldman G, Rueff B, Benhamou JP, Ropartz C. Heterozygous alpha-1-antitrypsin deficiency and cirrhosis in adults, a fortuitous association. *Lancet* 1975; 1 : 250-1.

Pongpaew P, Schelp FP. Alpha-1-protease inhibitor phenotypes and serum concentrations in Thailand. *Hum Genet* 1980; 54 : 119-24.

Rabinovitz M, Gavaler JS, Kelly RH, Prieto M, van Thiel DH. Lack of increase in heterozygous alpha-1-antitrypsin deficiency phenotypes among patients with hepatocellular and bile duct carcinoma. *Hepatology* 1992; 15 : 407-10.

Sharp HL. Alpha-1-antitrypsin: an ignored protein in understanding liver disease. *Semin Liver Dis* 1982; 2 : 314-28.

Sveger T. Liver disease in alpha-1-antitrypsin deficiency detected by screening of 200,000 infants. *N Engl J Med* 1976; 294 : 1316-21.

Tschesche HN, Haupt H, Schwick HG. Proteinase inhibitors of human plasma. In: Fritz H, Tschesche H, eds. Proceeding of the International Conference on Proteinase Inhibitors. Berlin. New York: de Gruyter, 1971: 1-22.