HEMOGLOBIN TYPING IN CHOLANGIOCARCINOMA

Somchai Insiripong[1], Sunthorn Thaisamakr[1] and Chularat Amatachaya[2],

[1]Department of Medicine, [2]Department of Clinical Pathology, Maharaj Nakhon Ratchasima Hospital, Nakhon Ratchasima 30000, Thailand

Abstract. Prevalence of thalassemias and/or hemoglobinopathies, particularly hemoglobin E, and cholangiocarcinoma were found more prominently in the lower part of the Northeast of Thailand as compared with the upper part of this region or any other area of the country. The aim of this study was to evaluate if there was coincident distribution or some relationship. Hemoglobin typing by the cellulose acetate method was performed in 111 cases of cholangiocarcinoma, mainly diagnosed by ultrasonography, compared with 146 normal controls. It was found that hemoglobin E trait and β-thalassemia trait were significantly higher in the former group.

INTRODUCTION

Cholangiocarcinoma (CCA) is a malignant tumor of the bile duct (Wanebo *et al*, 1989). It may be classified into 2 groups according to the sites where it arises. One is the peripheral type if it develops from small bile ducts within the liver. The other is the hilar type that originates from the major hepatic ducts near or at the juncture of the right and left hepatic ducts.

Compared with other regions of the world, cholangiocarcinoma is most frequently found in Thailand and it is responsible for onethird of all primary liver cancer, the most common malignant tumor in this country (Srivatanakul and Sontipong, 1979; Srivatanakul *et al*, 1988). Its geographic distribution in various regions of Thailand is quite different, most cases have occurred in the northeastern part where high endemicity of thalassemia and/or abnormal hemoglobins was found as well. When this particular region was arbitrarily divided into two areas, the upper and the lower, the latter had a higher prevalence, not only of hemoglobin E the so called "Hemoglobin E triangle": (Wasi *et al*, 1969; Wasi, 1981) but also of cholangiocarcinoma. On the contrary, opisthorchiasis which, in combination with nitrosamine ingestion (Bhamarapravati *et al*, 1978; Migasena and Changbumrung, 1974) was proposed to be a major responsible factor of this malignancy (Flavell, 1981; Kurathong *et al*, 1985) is more predominant in the upper than in the lower part of the Northeast region (Preuksaraj, 1982). From this point of view, opisthorchiasis with or without nitrosamine may not be the sole factor which plays a role in the etiology of this cancer. On the other hand, any relationship between cholangiocarcinoma and thalassemias or abnormal hemoglobins, should be taken into consideration. To verify this proposed hypothesis, hemoglobin typing in cases of cholangiocarcinoma and controls was performed and the frequencies of various thalassemias and/or hemogobinopathies in both groups were compared.

MATERIALS AND METHODS

Venous blood was taken from 111 patients with cholangiocarcinoma diagnosed by ultrasonography (Dhiensiri *et al*, 1988); some were confirmed by computerized tomography, exploratory laparotomy, biopsy or combination of these. Types of hemoglobin of these blood samples were identified by hemoglobin electrophoresis, using the cellulose acetate method. The amounts of hemoglobin A_2 and F were quantified by elution technique and alkaline denaturation methods, respectively.

The percentage of hemoglobin A_2 was used to differentiate hemoglobin E trait, hemoglobin E disease and β-thalassemia trait, *ie*:

$A_2 > 5\%$: β-thalassemia trait

$> 10\%$: Hb E trait

$> 60\%$: Hb E disease

Controls were patients randomly selected from those who attended the OPD clinic, Department of Medicine. Types of hemoglobin of the controls were identified with the same methods.

Statistical methods

Numbers of cases of various thalassemias and abnormal hemoglobins in both groups were compared, using X^2 test. Differences at $p< 0.05$ were considered statistically significant.

RESULTS

The patients consisted of 90 males and 21 females, age range was 38 to 77 years with 59 years as mean. Their ages, sexes and home places are shown in Table 1. Interpretation of hemoglobin typing in cholangiocarcinoma and in controls is shown in Table 2.

Table 1

Characteristics of the patients and control group.

	Cholangiocarcinoma	Control
Age range	38-77	17-86
Mean age	59	48
Male: female	90:21	61:85
Korat:non-Korat	61:50	127:19
Total	111	146

Among various kinds of thalassemia and/or hemoglobinopathy, only hemoglobin E trait and β-thalassemia trait were found significantly higher in cholangiocarcinoma patients than in controls.

DISCUSSION

Any relationship between malignancy and thalassemias and/or hemoglobinopathies is still inconclusive. Quattrin *et al* (1980) found a high frequency rate of malignancy in people with hemoglobin Lepore, while Miniero and Sarraco (1988) as well as Russo and Schilio (1987) found no relationship between homozygous β-thalassemia and cancer. Stricker *et al* (1986) found that hemoglobin S did not appear to exert a protective effect against childhood hematologic malignancies.

Regarding association between malignancy and hemoglobin E trait and β-thalassemia trait. Gupta *et al* (1988) found that overall incidence of hemoglobinopathies, including sickle cell trait, β-thalassemia trait, hemoglobin D trait and hemoglobin E trait was much higher in hematologic malignancies than in the general population.

In Thailand, Panich *et al* (1974) found that the incidence of hemoglobin E trait seemed to be slightly

Table 2

Hemoglobin typing interpretation.

	CCA	Control	X^2	p value	OR
Hb E trait	46	44	5.97	0.0145*	2.01
Hb E disease	5	11	0.05	0.8164	0.88
β-thal trait	16	9	7.61	0.0058*	3.42
Hb AEBart disease	1	2	0.34	0.7313	0.96
β-thal major	1	1	0.08	0.5722	1.92
Hb H disease	0	1	0.12	0.6610	0.00
β-thal Hb E	1	1	0.08	0.5722	1.92
Hb CS trait	1	0	0.10	0.3474	undefined
Normal	40	77			
Total	111	146			

* : statistically significant.

higher in people with malignant lymphoma than in the general population whereas β-thalassemia trait was not different. They suggested that a person with the hemoglobin E gene might be more prone to develop lymphoma than those without this mutant gene.

From our study hemoglobin E trait and β-thalassemia trait were found more frequently in cholangiocarcinoma than in controls. Others such as hemoglobin E disease, hemoglobin AEBart's disease, β-thalassemia major, hemoglobin H disease and β-thalassemia-hemoglobin E disease were not different between the two groups.

For cholangiocarcinoma, as with other malignancies, direct etiologic factor (s) are not simply identified. As far as we know, some contributing etiologic factors which had been shown to play an important role in induction of cholangiocarcinoma are opisthorchiasis, nitrosamines (Thamavit *et al*, 1978) and possibly gall stones (Bismuth and Malt, 1979); no report mentioning the role of hemoglobin E trait, β-thalassemia trait or any other thalassemia and/or hemoglobinopathy has previously appeared.

For hemoglobin E disease or other thalassemia major, the numbers of subjects were too small to be discussed.

The reasons why hemoglobin E trait and β-thalassemia trait are more frequently found in persons with cholangiocarcinoma, compared with controls, is not known. Possible explanations are proposed as follows. First there may be some genetic linkage.

In severe thalassemia, serum vitamin E, well known to have antioxidative action, is low, therefore free radicals including lipid peroxidation can be found; these have been suggested to be important causative agents in both the aging process and in several classes of disease including cancer and thalassemia (Chanarat, 1992).

Gall stones may be associated with cholangiocarcinoma (Bismuth and Malt, 1979). Red blood cells containing hemoglobin E are unstable to oxidants and have a slightly shortened survival as well as those of β-thalassemia (Wintrobe, 1981) resulting in chronic hemolysis and increased incidence of gall stones in severe thalassemias. But a raised incidence of gall stone in hemoglobin E trait or β-thalassemia trait has not been proven.

In case of chronic typhoid carrier state, Welton *et al* (1979) found that chronic infection of the gall bladder due to *Salmonella* might cause stone formation and cancer of the bile duct. But a relation between typhoid carrier state and hemoglobin E or β-thalassemia trait had not been proven.

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