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Risk factors for cholangiocarcinoma in a low risk Caucasian population

Summary

Objectives: Little is known about the etiology of cholangiocarcinoma, apart from its association with liver fluke infestation.
Methods: A case-control study was conducted in Athens and included six cholangiocarcinoma cases, 333 hepatocellular carcinoma cases and 360 controls with minor ailments or injuries. All subjects were interviewed and tested for hepatitis B and C infection. For all six cholangiocarcinomas, 97 hepatocellular carcinomas and 129 controls, sex steroids were determined in the blood.
Results: Hepatitis B or C viruses, tobacco smoking, alcohol drinking and diabetes mellitus were not related to cholangiocarcinoma, but history of thyroid disease was. Estradiol levels were higher among cholangiocarcinoma cases than among the other two groups.
Conclusions: This study provides evidence that endocrine and autoimmune factors are important in the etiology of cholangiocarcinoma.

Key-Words: Cholangiocarcinoma – Etiology – Estradiol – Thyroid disease – Liver disease.

Cholangiocarcinoma (CAC) originates from the intrahepatic bile ducts and is a rare form of primary liver cancer in all parts of the world, except in East/South-East Asia where it has been linked to infestation with *Clonorchis sinensis* and *Opisthorchis viverrini*[1,2]. Because of the rarity of CAC, few studies outside Asia have explored epidemiological aspects of this tumor[3,4]. There is clinical evidence that the tumor may complicate long-standing autoimmune conditions, notably ulcerative colitis and primary sclerosing cholangitis, but the data are inconclusive[5–7]. CAC may also follow exposure to thorotrast[8] and anabolic steroids[9]. In the course of a large case-control study of liver cancer in Greece[10–13], information and serum samples were also collected from cases of histologically confirmed CAC.

Materials and methods

In a four-year period between January 1995 and December 1998, a case-control study of primary liver cancer of any histological type was undertaken in Athens, Greece. During this period, 380 incident cases of primary liver cancer were admitted to three teaching hospitals in Athens[10–13]. Of these patients, 374 were diagnosed with hepatocellular carcinoma (HCC) and six with CAC. All six cases of CAC were Caucasian of Greek nationality and permanent residence, all were histologically confirmed, and all of them agreed to participate in the study. Controls were patients hospitalised for injuries, or eye, ear, nose, or throat conditions, that is, patients with non-cancer disorders usually requiring some minor operation. The protocol called for controls to be age and gender matched to the 333 HCC cases who agreed to participate. A total of 360 controls were included in the study. All cases and controls were interviewed in the hospital, using a standardised questionnaire. Among cases, blood sampling was done as soon as a strong suspicion was developed for an incident primary liver cancer. Among controls, blood samples were collected as soon as the eligible controls agreed to participate in the study. Sera were transferred on dry ice to the internationally certified Biomedicine Laboratories in Athens, Greece, for serological testing for hepatitis B virus (HBV) and hepatitis C virus (HCV) markers. Details about the study are given elsewhere[10,11]. Randomly chosen coded sera from 97 HCC cases, 129 controls, and six cases with CAC were also tested for estradiol, testosterone and sex hormone binding globulin (SHBG) in the same laboratory. Estradiol was measured using a chemiluminescence

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assay (Boehringer Mannheim, UK), whereas testosterone and SHBG were both measured using radioimmunoassays (DSL, Houston, Texas).

Results

Table 1 shows characteristics of the cases of CAC and the controls. No patient with CAC had evidence of chronic infection with hepatitis B or C virus, or of cirrhosis in contrast to the high proportions observed among HCC patients. There was no remarkable difference between CAC cases and controls with respect to socio-economic status, as reflected by years of schooling, tobacco smoking, excessive alcohol consumption or coffee drinking. There was also no evidence that diabetes mellitus is involved in the etiology of CAC, and the data was too sparse to allow an evaluation of a possible correlation between cholecystectomy for cholelethiasis with CAC. Four patients (all women) in the CAC series reported past thyroid disease, a proportion far higher than among the HCC cases or controls, even when gender differences among the three series were accounted for. We

	CAC cases	Controls	HCC cases
Gender			
Males	2 (33 %)	298 (83 %)	283 (85 %)
Age			
≥ 60	4 (67 %)	260 (72 %)	238 (71 %)
Range	36–72	24–96	31–93
School			
≥ 12 years	2 (33 %)	97 (27 %)	79 (24 %)
HBsAG			
Positive	0	12 (3 %)	209 (63 %)
Anti-HCV			
Positive	0	1 (0,3 %)	52 (16 %)
Cirrhosis			
Yes	0	0	131 (39 %)
Alcohol			
≥ 40 glasses/week	0 (0 %)	56 (16 %)	81 (24 %)
Smokers			
Ever smoker	3 (50 %)	234 (65 %)	235 (71 %)
Coffee consumers			
≥ 20 cups/week	1 (17 %)	90 (25 %)	67 (20 %)
Diabetes Mellitus			
Yes	0	39 (11 %)	60 (18 %)
Cholecystectomy			
Yes	1 (17 %)	25 (7 %)	45 (14 %)
Thyroid disease			
Yes	4 (67 %)	17 (5 %)	11 (3 %)

Table 1 Characteristics of six cases with cholangiocarcinoma (CAC), 360 controls and 333 hepatocellular carcinoma (HCC) cases in Athens, Greece

	CAC cases	Controls	HCC cases
Estradiol (pg/ml)			
Men	126.5 (46.5)	40.9 (1.0)	69.0 (6.0)
Women	53.8 (10.8)	25.5 (2.8)	32.8 (4.5)
Testosterone (ng/dl)			
Men	292.5 (227.5)	356.3 (10.6)	213.4 (15.9)
Women	37.0 (6.3)	26.8 (2.8)	32.1 (4.9)
SHBG* (nmol/L)			
Men	47.0 (12.0)	35.9 (1.1)	41.9 (2.3)
Women	61.0 (9.7)	59.2 (4.7)	78.5 (17.2)

* Sex hormone binding globulin

Table 2 Mean values (and standard errors) for specified hormone levels among two male and four male cholangiocarcinoma (CAC) patients, 112 male and 17 female controls, and 85 male and 12 female hepatocellular carcinoma (HCC) patients

searched the clinical records of the four women with CAC and history of thyroid disease. One of them, aged 36 at the diagnosis of CAC, had thyroid disease diagnosed at age 27; the second, aged 67 at the diagnosis of CAC, had thyroid disease diagnosed at age 37; the third, aged 70 at the diagnosis of CAC, had thyroid disease diagnosed at age 50; and the fourth, aged 72 at the diagnosis of CAC, had thyroid disease diagnosed at age 56. For the younger two of these women, there was information compatible with thyroid disease of autoimmune nature, whereas for the other two the information available was insufficient.

Table 2 shows the mean values (and standard errors) for estradiol, testosterone, and SHBG among the CAC patients, the controls and the HCC cases, by gender. We compared hormone levels in CAC cases with those among controls and HCC cases, in turn, by modeling the data through logistic regression, controlling for age and gender. The CAC cases had substantially higher levels of estradiol in comparison to controls, whereas no difference was evident with respect to testosterone or SHBG. Moreover, estradiol levels among men and women with CAC were also considerably higher compared with those among cases with HCC, even though the level of functional liver damage, as evaluated by prothrombin time and serum albumin level, was similar among CAC cases and among cases with HC.

Discussion

Very few studies among Caucasians have used an epidemiologic protocol to study risk factors for CAC and there is a paucity of documented findings. Although it is generally assumed that chronic infection with hepatitis B and C viruses do not cause CAC, we have been able to confirm this in the context of an epidemiologic investigation. Our results also indicate that neither alcohol nor tobacco smoking, both

of which have been shown to be related to HCC[11,14–16] are likely to play a role in the etiology of CAC, and this is in support of previous findings[17–19]. Cirrhosis of any form is associated with primary liver cancer, irrespective of the histology of the latter, but registry-based data are compatible with our finding that cirrhosis is less frequently associated with CAC than with HCC[4]. Observational studies also support the lack of a strong association of CAC with cirrhosis[20,21], and indicate that progression to CAC from primary sclerosing cholangitis is not predicted by the presence or severity of liver cirrhosis[17,19].

We have found evidence in this study that estradiol levels are elevated among both men and women with CAC, even in comparison with cases with HCC that have similar functional liver damage. This finding is compatible with an earlier report that tamoxifen inhibited the growth of human CAC, both *in vivo* and *in vitro*, suggesting that estradiol could be mitogenic for intrahepatic bile duct cells[22]. The higher incidence of CAC among men than among women in global statistics could be explained by a differential effect of estradiol depending on the levels of testosterone, a known anabolic factor.

Moreover, we found evidence indicating that, among women, thyroid disease, which frequently has autoimmune components, may be a risk factor for CAC, thus suggesting that such components may be operative in the pathogenesis of CAC. Recall bias can never be excluded in a case-control study, but is unlikely in this instance, because thyroid disease is not generally perceived as a risk factor of primary liver cancer of any type. On the other hand, a number of case reports have been published linking CAC to autoimmune disorders, including systemic lupus erythematosus[23], primary sclerosing cholangitis and ulcerative colitis[7], autoimmune hepatitis[24], as well as antiphospholipid syndrome[25]. Thyroid disease is common, frequently of autoimmune nature and has been associated with primary sclerosing cholangitis[26,27]. Thus, our finding of a very high co-existence of CAC with history of thyroid disease is plausible. The plausibility is increased by the apparent latency between thyroid disease and CAC and indicates that this tumor may be one of the few cancers with a dominant autoimmune pathogenesis, at least in its early stages. However, one cannot exclude environmental causal scenarios. It has been reported that environmental pollutants, like polychlorinated biphenyls (PCBs), can alter thyroid function[28–31] and as these pollutants are metabolised in the liver, they could be implicated in liver damage. The evidence, however, remains weak. In any case, the small number of cases in our study hinders definitive conclusions and these results should be viewed as preliminary, pending confirmation in additional or larger studies.

Zusammenfassung

Risikofaktoren für Cholangiokarzinom in einer kaukasischen Bevölkerung mit niedrigem Risiko

Fragestellung: Es ist wenig bekannt über die Ätiologie des Cholangiokarzinoms, ausser dem Zusammenhang mit einer Leberegelinfektion.

Methoden: Eine Fall-Kontroll-Studie mit sechs Fällen von Cholangiokarzinom, 333 von hepatozellulärem Karzinom und 360 Kontrollfällen mit kleineren Leiden und Verletzungen wurde in Athen durchgeführt. Alle Befragten wurden auf Hepatitis B und C getestet. Bei den sechs Cholangiokarzinom-Fällen, bei 97 Fällen mit hepatozellulärem Karzinom und bei 129 Kontrollfällen wurden die Geschlechtshormone im Blut gemessen.

Ergebnisse: Während zwischen Hepatitis B oder C, Rauchen, Alkohol, Diabetes Mellitus und dem Cholangiokarzinom kein Zusammenhang bestand, wurde ein solcher zwischen Schilddrüsenerkrankungen und dem Cholangiokarzinom festgestellt. Bei den Fällen mit Cholangiokarzinom war der Oestradiolwert höher als in den zwei anderen Fall-Gruppen.

Schlussfolgerungen: Diese Studie zeigt, dass endokrine und autoimmune Faktoren wichtig für die Ätiologie des Cholangiokarzinoms sind.

Résumé

Facteurs de risque de cholangicarcinome dans une population caucasienne à bas risque

Objectives: On connaît très peu dé choses sur l'étiologie du cholangiocarcinome hormis son association avec la douve du foie.

Méthodes: Une étude cas témoins, menée à Athènes, incluait 6 cas de cholangiocarcinome, 333 carcinomes hépatocellulaires et 360 témoins souffrant d'affections bénignes ou de traumatisme. Tous les sujets interviewés ont été testés pour l'hépatite B et C. Les stéroides sexuels ont été mesurés dans le sang des 6 cas de cholangiocarcinome, 97 carcinomes hépatocellulaires et 129 témoins.

Résultats: Les virus de l'hépatite B ou C, le tabagisme, la consommation d'alcool et le diabète sucré n'étaient pas associés au cholangiocarcinome, mais l'histoire de maladie thyroidienne l'était. Les taux d'estradiol étaient plus élevés parmi les cas de cholangiocarcinome que parmi les deux autres groupes.

Conclusions: Cette étude démontre l'importance des facteurs endocrines et auto-immuns pour l'étiologie du cholangiocarcinome.

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