2. Studies of Cancer in Humans

2.1 Descriptive studies

The association between liver fluke infection and the occurrence of cancer in humans has been reviewed extensively (Stewart, 1931; Higginson, 1955; Yamagata & Yaegashi, 1964; Gibson, 1971; Tansurat, 1971; Viranuvatti & Stitnimankarn, 1972; Schwartz, 1980; Flavell, 1981; Juttijudata *et al.*, 1984; Kim, 1984; Chan & Lam, 1987; Haswell-Elkins *et al.*, 1992a,b; Parkin *et al.*, 1993; Sithithaworn *et al.*, 1994).

2.1.1 *Opisthorchis viverrini*

All of the available studies are from Thailand, where there is substantial geographical variation in the prevalence of infection, increasing from the south to the north, the highest rates being observed in Khon Kaen Province in North-east Thailand (see section 1.3.1a). In incidence data from the national cancer registry, the highest frequency was observed in North-east Thailand in 1980–82 (Srivatanakul *et al.*, 1988) and again, especially in Khon Kaen Province, in 1988–91 (Vatanasapt *et al.*, 1993). In the earlier period, the proportionate incidence ratio was 3.1 (95% confidence interval [CI], 2.8–3.5) for cholangiocarcinoma and was 1.2 (95% CI, 1.1–1.4) for hepatocellular carcinoma (Srivatanakul *et al.*, 1988). In Khon Kaen Province around 1985, the age-standardized incidence rate of cholangiocarcinoma was 84.6 per 100 000 per year in men and 36.8 per 100 000 per year in women. Outside of Thailand, the incidence of cholangiocarcinoma shows little variation (range, 0.2–2.8 per 100 000 per year in men, and 0.1–4.8 per 100 000 per year in women) (Parkin *et al.*, 1993). Thus, the incidence in the area of highest incidence in Thailand is at least 40 times as high as that in the area of highest incidence elsewhere.

Within Khon Kaen Province, during the period 1985–88, Vatanasapt *et al.* (1990) observed the highest incidence and mortality rates of liver cancer in three adjacent districts; studies in two of the districts had shown high prevalences of infection and heavy infection (Upatham *et al.*, 1984). Subsequently, Sriamporn *et al.* (1993) showed that there was no difference in the overall prevalence of infection between the districts of highest and lowest incidence of liver cancer within the Province during the period 1988–90; however, 9% of 331 subjects from randomly selected villages in the district of highest incidence had > 10 000 fluke eggs/g of stool, while only 3.7% of 296 subjects in villages in the district of low incidence had the same level of infection.

Srivatanakul *et al.* (1991a) carried out a correlation analysis of liver cancer incidence, titre of antobodies to *O. viverrini* and faecal egg count (determined in healthy volunteers who

had been born and resided in the area) in five regions with different frequencies of the two main histological types of liver cancer: Chiang Mai in the north, Nakon Ratchasima and Ubon Ratchathani in the north-east (but not in Khon Kaen Province), Bangkok in the centre and Songkhla in the south. The correlation between the incidence of cholangiocarcinoma and the proportion of subjects with an antibody titre $\geq$ 1:40 was 0.98 ($p = 0.004$), and that with faecal egg count was 0.53 ($p = 0.35$). For hepatocellular carcinoma, which showed little geographical variation in incidence, the correlations were –0.37 ($p = 0.54$) and 0.02 ($p = 0.96$), respectively. [The weaker association between cholangiocarcinoma and faecal egg count may reflect the introduction of effective therapy; antibody titre is thought to provide a more valid indicator of past infection, but cross-reactivity with other parasites common in the region may have been involved.]

These studies are summarized in Table 1.

2.1.2 *Opisthorchis felineus*

In the T'umen' region in western Siberia (an area of *O. felineus* endemicity), Shain (1971) related the prevalence of infected people in four subregions as reported by local health centres with the incidence of liver cancer observed in the same period, 1960–69. The correlation computed by the Working Group from the tabulated data was [0.98; $p < 0.05$]. A similar analysis in seven cities within one of the regions confirmed this correlation [0.77]. No information was given on the relative frequency of histological types.

2.2 Case reports and case series

2.2.1 *Opisthorchis viverrini*

All of the available reports are from Thailand. The earliest case reports are of a papillary adenocarcinoma of the liver and an adenocarcinoma of the bile duct (Viranuvatti & Mettiyawongse, 1953) and a retention cyst of the liver caused by opisthorchiasis associated with carcinoma of the liver (Viranuvatti *et al.*, 1955); *O. viverrini* infection was detected at autopsy in each case. Subsequent case series are summarized in Table 2. Among patients from the area in which *O. viverrini* is endemic, cases of cholangiocarcinoma outnumber cases of hepatocellular carcinoma, in contrast to other series.

Cancers other than of the liver have been reported in association with this infection, but no particular type has predominated (Koompirochana *et al.*, 1978; Pungpak *et al.*, 1985).

2.2.2 *Opisthorchis felineus*

Three studies on the presence of *O. felineus* infection in liver cancer cases were conducted in western Siberia (Table 3). One of the regions, T'umen', is reported to be an area of high endemicity. The prevalence of infection in 250 histologically verified cases of liver cancer was 52% in the study of Shain *et al.* (1971). The prevalence of infection in 44 cases of liver cancer detected in 657 autopsies performed in the same region was 95% (Glumov *et al.*, 1974). The first study also reported a higher frequency of cholangiocarcinoma among infected liver cancer cases and a difference in the sex ratios between the two main histological types [no information was provided about the sex ratio of infection].

Table 1. Descriptive studies of *Opisthorchis viverrini* and liver cancer in Thailand

Reference	Area and period of study	Details of cases of liver cancer			Measure of exposure to *O. viverrini*	Number of geographical units	Association	Comments
		Deaths or incidence	Type	Number				
Srivatanakul *et al.* (1988)	Whole country, 1980–82	Incidence	Liver cancer CCA HCC	3820 523 779	–	10 9 9	Highest PIR for liver cancer (men, 2.0; 95% CI, 1.9–2.2; women, 2.7; 95% CI, 2.4–3.0) observed in Khon Kaen Province in North-east Thailand. Highest PIR (3.1, 95% CI, 2.8–3.5) for CCA observed in North-east Thailand. Corresponding PIR for HCC was 1.2 (95% CI, 1.1–1.4).	
Vatanasapt *et al.* (1993)	Four population-based cancer incidence registries, 1988–91	Incidence	Liver cancer	4314	–	4	Highest incidence for CCA in Khon Kaen Province in North-east Thailand	
Vatanasapt *et al.* (1990)	Khon Kaen Province, 1985–88	Incidence Deaths	Liver cancer Liver cancer	1338 NR	–	20	Highest incidence and mortality rates in three adjacent districts (Chonnabot, Nong Rua and Muncha Khiri), in which other studies showed high prevalences of infection and heavy infection	Rate of total cancers in these areas very high
Sriamporn *et al.* (1993)	Districts with highest (Chonnabot) and lowest (Ban Phang) incidence of liver cancer in Khon Kaen Province, 1988–90	Incidence	Liver cancer	140	Eggs/gram in stool samples from 627 subjects aged ≥30 from randomly selected villages in each district	2	No difference in overall prevalence of infection; 9% of subjects from district in high-incidence area had > 10 000 eggs/g, compared with 3.7% in the other district	No significant difference in age and sex distribution of subjects
Srivatanakul *et al.* (1991a)	Five areas with different frequencies of CCA and HCC, 1980–82, 1983–87, 1988, depending on area	Incidence	CCA HCC		Antibody titre and faecal egg count in about 100 volunteers aged 30–40 in each area	5	Positive correlation between proportion of subjects with antibody titre ≥1:40 and CCA (r = 0.98, p = 0.004). Correlation between eggs/g and CCA was 0.53 (p = 0.35). Corresponding correlations with HCC –0.37 (p = 0.54) and 0.02 (p = 0.96)	No strong or significant correlations between CCA and HBV infection, prevalence of HBsAg carriers, and aflatoxin levels in serum or urine

CCA, cholangiocarcinoma; HCC, hepatocellular carcinoma; PIR, proportionate incidence ratio; CI, confidence interval; HBV, hepatitis B virus; HBsAg, hepatitis B surface antigen; NR, not reported

Table 2. Case series of patients with liver cancer associated with *Opisthorchis viverrini* infection in Thailand

Reference	Patients specified as coming from endemic area	Period of study	Cases				
			Method of ascertainment	Type	Number	*O. viverrini* infection	
						No.	%
Bhamrapravati & Virranuvatti (1966)	No	1960–62	Liver biopsy	HCC	251	5	2
				CCA	61	11	18
		1959–61	Autopsy	HCC	33	0	0
				CCA	14	11	79
Chainuvati *et al.* (1976)	Yes	NR	NR	Adenocarcinoma of cystic duct	4	3[a]	75
Koompirochana *et al.* (1978)	No	1954–74	Autopsy	HCC	266[b]	9	3.4
				CCA	108[b]	67	62
Sonakul *et al.* (1978)	No	17 years	Autopsy	HCC	9	From case series with *O. viverrini*	
				CCA	67		
	Yes	3 years	Autopsy	HCC	3	3	100
				CCA	8	8	100
Stitnimankarn *et al.* (1978)	Yes	NR	Liver biopsy	CCA	11	11	100
Pungpak *et al.* (1985)	No	1982–84	Autopsy, liver biopsy, surgery, ascitic fluids	Adenocarcinoma of liver	16	From case series with severe *O. viverrini*	
Riganti *et al.* (1989)	Yes	1969–88	Autopsy	Adenocarcinoma of bile duct	8	From case series with *O. viverrini*	
				HCC	2		

NR, not reported; HCC, hepatocellular carcinoma; CCA, cholangiocarcinoma

[a]By stool examination; all were found to have infection when the ducts were examined histologically.

[b]Combining cases reported to have *O. viverrini* infection and those reported to be without the fluke

Table 3. Prevalence of *Opisthorchis felineus* in case series of liver cancer in western Siberia in the Russian Federation

Reference	Region	Endemicity	Cases				Results
			Method of ascertainment	Total no.	*O. felineus* infection		
					No.	%	
Shain *et al.* (1971)	T'umen'	High	Clinical	250	130	52	Sex ratio (M/F) in uninfected same as expected from literature, i.e. 2–6; sex ratio in infected was reversed [figures not given]. Cancers in uninfected patients mainly HCC; those in infected patients CCA: 4–5 times more frequent than HCC
Glumov *et al.* (1974)	T'umen'	High	Autopsy	44	42	95	35/44 CCA, frequency in infected not given. Prevalence of liver cancer at autopsy 6.7%; 0.7% in another pathology department
Iablokov *et al.* (1980)	Tomsk	Intermediate	Autopsy	103	7	7	In the whole series, 54% HCC and 46% CCA. Four infected cases had CCA; 3 had HCC.

HCC, hepatocellular carcinoma; CCA, cholangiocarcinoma

In a similar study conducted in a region of intermediate endemicity, 7 liver cancers out of 103 detected at autopsy were infected with *O. felineus* (Iablokov *et al.*, 1980). Similar proportions of cases of cholangiocarcinoma (4/47) and hepatocellular carcinoma (3/56) were infected.

2.2.3 *Clonorchis sinensis*

The earliest case reports of primary liver cancer concerned Chinese subjects (Watson-Wemyss, 1919; Bentham, 1920; Nauck & Liang, 1928; Ch'in *et al.*, 1955). Subsequent case series, from Hong Kong and the Republic of Korea and among Asian subjects in the USA, are summarized in Table 4. Cases have also been described in immigrants to North America from China (Schwartz, 1986; Colquhoun & Visvanathan, 1987) and Laos (Drinka & Sheehy, 1985; Sher *et al.*, 1989; Ona & Dytoc, 1991). The only other population in which cases have been reported is that of Japan (Nakashima *et al.*, 1977).

2.3 Case–control studies

2.3.1 *Opisthorchis viverrini*

Kurathong *et al.* (1985) assessed the prevalence of cholangiocarcinoma and hepatocellular carcinoma during 1981–83 in 551 (47%) patients from the north-east (49.8% of those attending a hospital in Bangkok) who agreed to provide stool specimens, on the basis of which they were characterized for the presence of *O. viverrini* eggs. All 551 were screened for hepatobiliary tract diseases. Nineteen of 25 cases of cholangiocarcinoma and 9 of 12 of hepatocellular carcinoma had ova in the stools. The cases were diagnosed by a variety of methods, including ultrasound biopsy and hepatic angiography. The crude prevalence odds ratios were [1.3 (0.5–3.6)] for cholangiocarcinoma and [1.3 (0.3–4.7)] for hepatocellular carcinoma. [Use of controls with other hepatobiliary disease may have biased the results.]

A hospital-based case–control study of cholangiocarcinoma (Parkin *et al.*, 1991) and hepatocellular carcinoma (Srivatanakul *et al.*, 1991b) was carried out in Thailand, in which 103 cholangiocarcinoma patients and 65 hepatocellular carcinoma patients living in and originating from North-east Thailand were recruited in 1987–88 from among patients whose disease was diagnosed sequentially in three hospitals. One control was matched to each case for sex, age (within five years), residence and hospital of recruitment. Controls were selected from among patients affected by a variety of non-malignant diseases, considered not to be related to the consumption of alcohol or tobacco. Infection with *O. viverrini* was assessed in terms of an increase in titre of antibodies to *O. viverrini* in serum as observed by ELISA (Srivatanakul *et al.*, 1985). For cholangiocarcinoma, the matched estimate of the odds ratio obtained from the final multivariate model, including adjustment for consumption of 'sticky' rice and betel-nut chewing, was 5.0 (95% CI, 2.3–11.0). No association was seen with chronic carriage of hepatitis B virus nor with recent aflatoxin intake (Parkin *et al.*, 1991). *O. viverrini* infection was not significantly associated with the risk of developing a hepatocellular carcinoma. The observed odds ratio was 1.7 (0.8–3.7). In a multivariate analysis, there was a strong association with chronic carriage of hepatitis B virus (Srivatanakul *et al.*, 1991b).

Haswell-Elkins *et al.* (1994a) conducted a cross-sectional population-based survey in 1990–91 of subjects aged 25 or more from 46 villages in two districts of Khon Kaen Province

Table 4. Case series of patients with cancer of the liver associated with Chlonorchis sinensis infection

Reference	Location	Period of study	Cases				
			Method of ascertainment	Type	No.	*C. sinensis* infection	
						No.	%
Hou (1956)	Hong Kong	7 years	Autopsy	Adenocarcinoma (21) and mixed type of intrahepatic second-order bile duct tumours	30	30[a]	100
Belamaric (1973)	Hong Kong	1961–66	Autopsy	Adenocarcinoma of intrahepatic bile duct	19	18	95
Chou & Chan (1976)	Hong Kong	1964–73	Autopsy	CCA	50	46	92
Purtilo (1976)	Hong Kong	NR	Autopsy	CCA	7	From series of subjects with *C. sinensis* infection	
				HCC	10		
Ho (1980)	Hong Kong	Before 1976[b]	Autopsy	Mucoepidermoid carcinoma of the liver	2	0	0
Koo *et al.* (1982)	Hong Kong	1976–80	Laparotomy	Mucoepidermoid carcinoma of the bile duct	3	3	100
Kim *et al.* (1974)	Republic of Korea	1962–72	Autopsy				
	Seoul			HCC	339	28	8.3
				CCA	33	8	24.2
	Pusan			HCC	84	15	17.9
				CCA	21	13	61.9
Choi *et al.* (1988)	Republic of Korea	7 years	Surgery	CCA	16	10	62.5
Choi *et al.* (1989)	Republic of Korea	4 years	Surgery	CCA	20	From series of subjects with *C. sinensis* infection	
				HCC	4		
Strauss (1962)	USA, Asian subjects	1945–60	Surgery	Hepatomas	5	From series of subjects with *C. sinensis* infection	

NR, not reported; CCA, cholangiocarcinoma; HCC, hepatocellular carcinoma

[a]Clonorchiasis; 28 (93%) cases were found to have flukes in the bile duct.

[b]Koo *et al.* (1982)

and 39 villages in Maha Sarakham Province, within the endemic area of *O. viverrini* infection in North-east Thailand. Stool specimens were obtained from 7727 subjects (participation rate, 72%) in Khon Kaen Province and 4585 subjects (participation rate, 79%) in Maha Sarakham Province after a health education programme about liver fluke infection. A 15% random sample of 1807 uninfected and lightly infected (< 3000 fluke eggs/g) subjects and all subjects with higher intensities of infection were invited to undergo an ultrasound examination. Among the 78% of subjects who complied, 44 had evidence of cholangiocarcinoma without overt symptoms. In nine of these, the diagnosis was corroborated by endoscopic retrograde cholangiopancreatography; a further six who died before they could undergo the procedure or who declined it were strongly suspected to have cholangiocarcinoma. Thus, there was a total of 15 cases, seven in patients who died with jaundice and hepatomegaly in 1991–92. Among 410 uninfected subjects, one case occurred. The multivariate prevalence odds ratios, accounting for age, sex and district of residence, were 1.7 (95% CI, 0.2–16.3) for subjects with up to 1500 fluke eggs/g, 3.2 (0.4–30) for subjects with 1501–6000 eggs/g and 14 (1.7–119) for more heavily infected subjects.

2.3.2 *Clonorchis sinensis*

In a consecutive series of 1484 autopsies in a single hospital in Hong Kong during the period 1964–66, clonorchiasis was found on gross examination in 11 of 17 (65%) cases of cholangiocarcinoma and in 24 of 83 (29%) cases of hepatocellular carcinoma. The expected proportions infected, on the basis of the whole series and adjusted for age and sex, were 38 and 35%, respectively. [The odds ratios, adjusted for age and sex, calculated by the Working Group, were 3.1 (95% CI, 1.1–8.4) for cholangiocarcinoma and 0.73 (0.45–1.2) for hepatocellular carcinoma] (Gibson, 1971).

Kim *et al.* (1974) studied records of autopsy and surgical specimens from one hospital in an area of low prevalence of *C. sinensis* (Seoul) and one hospital in an area of high prevalence (Pusan) in the Republic of Korea during the period 1961–72. In the area of low prevalence, a total of 386 histologically proven cases of primary liver cancer were identified among 1447 subjects with liver disease, and in the area of high prevalence, there were 109 cases of primary liver cancer among 396 subjects with liver disease. *C. sinensis* infection was determined by examination of liver tissue or stool samples. Comparison of cases of liver cancer with subjects with liver disease in whom cancer was not found showed a weak positive association between the cancer and *C. sinensis* infection [odds ratio, 1.7; 95% CI, 1.2–2.3]. The corresponding odds ratio for cholangiocarcinoma, based on 54 cases, was [6.5 (95% CI, 3.7–12)] and that for hepatocellular cancer, based on 423 cases, was [1.2, 0.80–1.7].

In Pusan, Republic of Korea, one of the areas of highest prevalence of *C. sinensis* infection, the occurrence of clonorchiasis was determined in stool specimens from 206 of a consecutive series of 368 cases of primary liver carcinoma diagnosed mainly in two hospitals during the period 1963–74 (Chung & Lee, 1976). [The Working Group noted that as one of these hospitals had been included in the study of Kim *et al.* (1974), there is some overlap with that study.] The control series comprised 559 subjects admitted to these hospitals with diseases other than of the liver; again, the presence of clonorchiasis was determined from stool specimens [no further details]. The crude odds ratio for cholangiocarcinoma, based on 36 cases, was [6.0 (95% CI, 2.8–13)]; the odds ratio was unchanged after adjustment for age

and sex. The crude odds ratio for hepatocellular carcinoma, based on 170 cases, was 1.1 (95% CI, 0.65–1.7).

These studies are summarized in Table 5.