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Epidemiological characteristics and risk factors of hepatocellular carcinoma

CHIEN-JEN CHEN,* MING-WHEI YU*† AND YUN-FAN LIAW‡

Graduate Institute of Epidemiology and †Department of Public Health, College of Public Health, National Taiwan University and ‡Liver Research Unit, Chang Gung Memorial Hospital, Taipei, Taiwan, Republic of China

Abstract Hepatocellular carcinoma (HCC) is one of the major cancers in the world. There is a striking variation in HCC incidence rates between various countries, with a highest-to-lowest ratio of 112.5 for males and 54.7 for females. The high-risk populations are clustered in sub-Saharan Africa and eastern Asia. The male-to-female ratio for HCC ranges from < 1 to 6.4 and mostly from 2 to 4. There exist significant variations in the incidence of HCC among different ethnic groups living in the same area and among migrants of the same ethnic groups living in different areas. The age curves of HCC are significantly different in various countries, suggesting variability in exposure to risk factors. Chronic carriers of hepatitis B and C viruses (HBV and HCV, respectively) have an increased risk of HCC. The relative and attributable HCC risk of HBV and HCV carrier status varies in different countries. There exists a synergistic interaction on HCC between the two viruses. Aflatoxin exposure, cigarette smoking, heavy alcohol consumption, low vegetable intake, inorganic arsenic ingestion, radioactive thorium dioxide exposure, iron overload and the use of oral contraceptives and anabolic steroids have been documented as HCC risk factors. Recent molecular epidemiological studies have shown that low serum retinol levels as well as elevated serum levels of testosterone, *neu* oncoprotein and aflatoxin B_1-albumin adduct are associated with an increased HCC risk. There is a synergistic interaction on HCC between chronic HBV infection and aflatoxin exposure. Familial aggregation of HCC exists and a major susceptibility gene of HCC has been hypothesized. Patients of some genetic diseases are at an increased risk of HCC. The genetic polymorphisms of cytochrome P450 2E1 and 2D6 and arylamine *N*-acetyltransferase 2 are associated with the development of HCC. A dose–response relationship between aflatoxin exposure and HCC has been observed among chronic HBV carriers who have null genotypes of glutathione *S*-transferase M1 or T1, but not among those who have non-null genotypes. Human hepatocarcinogenesis is a multistage process with the involvement of a multifactorial aetiology. Gene–environment interactions are involved in the development of HCC in humans.

Key words: aetiology, epidemiology, gene–environment interaction, hepatocellular carcinoma.

INTRODUCTION

Hepatocellular carcinoma (HCC) is a highly malignant disease with an extremely poor prognosis. It is a major cancer with approximately one million deaths annually in the world.[1] For the effective prevention of HCC, it is essential to explore risk factors associated with the disease. The occurrence of HCC varies in time, place and person. These epidemiological characteristics of a disease may provide clues for the formation of hypotheses regarding risk factors for the disease. The epidemiological hypotheses may be further elucidated through case–control and cohort studies. The present paper describes the epidemiological characteristics and multiple risk factors of HCC with special emphasis on studies performed in Taiwan.

EPIDEMIOLOGICAL CHARACTERISTICS

International and intranational variation

Based on the incidence data of liver cancer registered in different areas in the world,[2] there was a striking difference of the age-adjusted incidence of liver cancer in different countries in 1983–87. As shown in Table 1, the ratio between the highest and lowest age-adjusted

Correspondence: Professor C-J Chen, Graduate Institute of Epidemiology, College of Public Health, National Taiwan University, 1 Jen-Ai Road Section 1, Taipei 10018, Taiwan, Republic of China. Email: < cjchen@ntumc1.mc.ntu.edu.tw >

Table 1 International comparison of age-adjusted incidence rates (100 000) of liver cancer

Country, region	Male	Female	Ratio
Thailand, Khon Kaen	90.0	38.3	2.4
China, Qidong	89.9	24.5	3.7
Mali, Bamako	47.9	21.4	2.2
Japan, Osaka	41.5	9.7	4.3
Hong Kong	39.2	9.6	4.1
Gambia	36.0	12.1	3.0
Singapore (Chinese)	26.8	7.0	3.8
Philippines, Manila	23.7	8.0	3.0
Italy, Trieste	14.5	2.5	5.8
Switzerland, Geneva	9.8	1.8	5.4
France, Bas Rhin	8.9	1.4	6.4
Spain, Basque County	8.2	2.6	3.2
Peru, Trujillo	7.4	5.1	1.5
Sweden	4.5	2.6	1.7
US, SEER (Black)	4.2	1.4	3.0
UK, SE Scotland	3.3	1.2	2.8
Germany, Saarland	4.0	1.6	2.5
Canada	2.6	1.0	2.6
Australia, Victoria	2.5	0.8	3.1
US, SEER (White)	2.4	1.1	2.2
Iceland	2.4	1.7	1.4
Paraguay, Ascucion	1.1	1.5	0.7
Ireland, Southern	1.1	1.1	1.1
Netherlands, Maastricht	0.8	0.7	1.1
Highest/lowest ratio	112.5	54.7	

Data taken from *Cancer Incidence in Five Continents, Volume VI.*[2]

Table 2 Intranational variation of age-adjusted incidence rates (per 100 000) of liver cancer in selected countries

Country	Highest (area)	Lowest (region)	Ratio
Male			
Thailand	90.0 (Khon Kaen)	19.8 (Chiang Mai)	4.5
China	89.9 (Qidong)	23.6 (Tianjin)	3.8
Japan	41.5 (Osaka)	11.9 (Yamagata)	3.5
Italy	14.5 (Trieste)	5.9 (Florence)	2.5
Switzerland	9.8 (Geneva)	4.8 (St Gall)	2.0
France	8.9 (Bas Rhin)	2.2 (Tarn)	4.0
Spain	8.2 (Bosque Country)	4.1 (Zaragoza)	2.0
UK	3.3 (SE Scotland)	1.4 (Oxford)	2.4
Canada	3.5 (Quebec)	1.3 (Newfoundland)	2.7
Australia	2.5 (Victoria)	1.3 (South)	1.9
Female			
Thailand	38.3 (Khon Kaen)	10.4 (Chiang Mai)	3.7
China	24.5 (Qidong)	8.7 (Tianjin)	2.8
Japan	9.7 (Osaka)	3.8 (Yamagata)	2.6
Italy	4.7 (Latina)	1.5 (Romagna)	3.1
Switzerland	1.8 (Geneva)	0.9 (Vaud)	2.0
France	1.4 (Bas Rhin)	0.4 (Isere)	3.5
Spain	2.8 (Zaragoza)	1.7 (Tarragona)	1.6
UK	1.4 (NE Scotland)	0.6 (Birmingham)	2.3
Canada	1.3 (Quebec)	0.5 (Newfoundland)	2.6
Australia	0.8 (Victoria)	0.4 (Tasmania)	2.0

Data taken from *Cancer Incidence in Five Continents, Volume VI.*[2]

incidence rates in the world was 112.5 (90.0 /100 000 in Khon Kaen *vs* 0.8 /100 000 in Maastricht) for males and 54.7 (38.3 /100 000 in Khon Kaen *vs* 0.7 /100 000 in Maastricht) for females. The areas of highest liver cancer incidence rates are located in sub-Saharan Africa and eastern Asia, including Khon Kaen, Qidong, Bamako, Osaka, Hong Kong, Gambia, Singapore and Manila. The significant difference in liver cancer incidence among various countries may be attributable to differences in their ethnic composition and natural and sociocultural environments. The male-to-female ratio of age-adjusted liver cancer incidence rates ranged from 0.7 (Ascucion, Paraguay) to 6.4 (Bas Rhin, France) but was mostly between 2 and 4. The gender difference may be due to discrepancies in the hepatitis B surface antigen (HBsAg) carrier rate, lifestyles, occupational exposure and hormone status between males and females.

In addition to the significant international variation, there were also significant intranational differences in age-adjusted incidence rates of HCC.[2] In the 10 countries included in Table 2, the ratios between highest and lowest age-adjusted incidence rates within the countries ranged from 1.9 to 4.5 for males and from 1.6 to 3.7 for females. The highest ratio was observed in Thailand for both males and females. This discrepancy suggests that host susceptibility and/or exposures to risk factors may be different in different areas of the same country. For example, the strikingly high mortality rate among residents in the Penghu Islets and the endemic area of blackfoot disease in Taiwan may be attributable to heavy exposure to aflatoxin and ingested inorganic arsenic, respectively.[3]

Ethnic and migrant variation

The difference in incidence rates of a given disease among different ethnic groups living in the same area suggests that specific ethnic background, either genetic or cultural, may play a more important role in the determination of the disease than common living environments shared by various ethnic groups. Table 3 shows the ethnic variation in age-adjusted incidence rates of liver cancer in Los Angeles, Hawaii, Singapore, Kuwait and New Zealand.[2] The ethnic difference was more striking in Los Angeles and New Zealand than in the other three areas. The highest-to-lowest ratio was 8.7 for males and 5.1 for females in Los Angeles and 5.0 for males and 3.3 for females in New Zealand. There was a significant difference in age-adjusted liver cancer incidence rates among Chinese, Filipinos and Japanese in Los Angeles, but not in Hawaii.

The difference in incidence rates of a given disease among migrants of the same ethnic group living in different areas suggests that environmental factors rather

Table 3 Ethnic difference in age-adjusted incidence rates (per 100 000) of liver cancer

Region	Ethnicity	Male	Female
Los Angeles	Korean	20.1	3.9
	Chinese	14.6	4.6
	Filipino	6.8	1.8
	Black	6.1	2.1
	Hispanic White	4.8	1.7
	Japanese	4.2	2.2
	Other White	2.3	0.9
	Highest/lowest ratio	8.7	5.1
Hawaii	Hawaiian	8.1	1.7
	Chinese	6.5	2.2
	Japanese	6.4	2.5
	Filipino	6.2	2.2
	White	2.5	1.2
	Highest/lowest ratio	3.2	1.5
Singapore	Chinese	26.8	7.0
	Malay	13.2	6.3
	Indian	9.4	4.6
	Highest/lowest ratio	2.8	1.3
Kuwait	Non-Kuwaitis	14.7	5.2
	Kuwaitis	7.2	2.4
	Highest/lowest ratio	2.1	2.1
New Zealand	Maori	11.1	3.0
	Non-Maori	2.2	0.9
	Highest/lowest ratio	5.0	3.3

Data taken from *Cancer Incidence in Five Continents, Volume VI.*[2]

Table 4 Migrant comparison of age-adjusted incidence rates (per 100 000) of liver cancer

Ethnicity	Region*	Male	Female
Chinese	China, Qidong	89.9	24.5
	Hong Kong	39.2	9.6
	China, Shanghai	30.6	10.7
	Singapore	26.8	7.0
	China, Tianjin	23.6	8.7
	Los Angeles	14.6	4.6
	Hawaii	6.5	2.2
	Highest/lowest ratio	13.8	11.1
Japanese	Osaka	41.5	9.7
	Hiroshima	28.2	7.5
	Yamagata	11.9	3.8
	Hawaii	6.4	2.5
	Los Angeles	4.2	2.2
	Highest/lowest ratio	10.0	4.4
Filipino	Manila	23.7	8.0
	Rizal	20.7	8.3
	Los Angeles	6.8	1.8
	Hawaii	6.2	2.2
	Highest/lowest ratio	3.8	4.4
Jews	Africa or Asia	3.1	1.4
	Europe or America	2.7	1.1
	Israel	1.8	0.4
	Highest/lowest ratio	1.7	3.5

*Living areas for Chinese, Japanese and Filipino; birth areas for Jews.

Data taken from *Cancer Incidence in Five Continents, Volume VI.*[2]

than ethnic background may play a major role in the determination of the disease. Table 4 illustrates migrant variations in age-adjusted incidence rates of liver cancer for Chinese, Japanese, Filipinos and Jewish people.[2] The highest-to-lowest ratio among Chinese was 13.8 for males and 11.1 for females. Migrants living in Los Angeles and Hawaii had a lower liver cancer incidence rate than those of the same ethnic group living in Asia. Jewish people born in Africa or Asia had the highest incidence of liver cancer compared with those who were born in Europe, America or Israel, exhibiting ratios of 1.7 for males and 3.5 for females. The migrant difference was observed for both males and females. The ethnic and migrant variation in age-adjusted incidence rates of liver cancer suggests that both ethnic and environmental factors are important determinants.

Temporal variation

Based on the liver cancer incidence rates reported by the International Agency for Research on Cancer,[2,4–6] the secular trend of age-adjusted incidence rates of liver cancer was quite different in different areas. As shown in Table 5, the liver cancer incidence rates in Osaka, Japan, increased rapidly from 1.5 to 41.5 /100 000 for males and from 0.4 to 9.7 /100 000 for females from 1968 to 1987. The trend of an increase in the incidence of liver cancer was consistent with that reported previously.[7] The incidence rates of liver cancer in males and females in Hong Kong, Sweden and Bas Rhin, France, increased steadily, albeit showing a lesser increase, during the same period of time.[2,4–6] The increase in incidence rates may be attributable to either an improvement in diagnosis or an increased exposure to risk factors. While the age-adjusted liver cancer incidence rates in Shanghai and Geneva remained almost unchanged, those in Singapore decreased significantly for males, from 34.2 to 26.8 /100 000, and also for females, from 8.0 to 7.0 /100 000. The decrease in incidence rates may be due to the decrease in exposures to risk factors. An eight-fold increase in the incidence of HCC has been reported in Florence, Italy from 1958 to 1982,[8] but a constant mortality rate from primary liver cancer was observed in England and Wales from 1975 to 1992.[9] The trend of increasing mortality

Table 5 Secular trend of age-adjusted incidence rates (per 100 000) in selected regions, 1968–1987

Region	Sex	Year 1968–1972	1973–1977	1978–1982	1983–1987
Japan, Osaka	Male	1.5	5.6	31.9	41.5
	Female	0.4	1.2	7.8	9.7
Hong Kong	Male	—	34.3	32.3	39.2
	Female	—	8.9	7.4	9.6
Shanghai	Male	—	31.7	34.4	30.6
	Female	—	9.1	11.6	10.7
Singapore, Chinese	Male	34.2	32.2	31.6	26.8
	Female	8.0	7.1	7.2	7.0
Switzerland, Geneva	Male	9.4	9.7	10.2	9.8
	Female	1.4	1.3	1.5	1.8
France, Bas Rhin	Male	—	4.9	6.9	8.9
	Female	—	0.7	1.2	1.4
Sweden	Male	2.9	3.4	4.7	4.5
	Female	1.4	1.8	2.7	2.6

Data taken from *Cancer Incidence in Five Continents, Volume III–VI.*[2,4-6]

Table 6 Comparison of age-specific incidence rates (per 100 000) of liver cancer in selected regions

Age	Qidong	Khon Kaen	Bamako	Osaka	Hong Kong	Gambia
10–14	0.3 >	0.0	1.7 >	0.2	0.9 <	1.5
15–19	1.1 >	0.4	4.8 >	0.1	1.1 <	2.0
20–24	7.4 >	1.0	6.8 >	0.3	1.7 <	7.4
25–29	40.4 >	6.4	17.1 >	0.6	5.0 <	28.1
30–34	114.1 >	16.2	28.6 >	1.6	12.1 <	38.6
35–39	172.3 >	35.6	58.1 >	5.5	20.5 <	44.2
40–44	211.2 >	70.5	78.5 >	14.4	46.7 <	55.8
45–49	244.1 >	125.4	108.3 >	33.4	66.7 <	94.3
50–54	221.8 <	250.0	99.0 <	109.2	93.1 >	58.2
55–59	267.6 <	373.6	117.1 <	181.1	123.6 >	84.3
60–64	206.0 <	404.3	184.7 <	217.1	155.1 >	78.8
65–69	207.2 <	427.9	109.7 <	234.7	181.7 >	64.8
70–74	149.6 <	482.5	306.1 >	246.3	196.8	—
75–79	133.3 <	387.1	133.1 <	237.1	206.1	—
ASR	89.9	90.0	47.9	41.5	39.2	36.0

ASR, age-adjusted rate.

Data taken from *Cancer Incidence in Five Continents, Volume VI.*[2]

rates from liver cancer in Taiwan was more striking in males than in females.[10] This gender difference in a secular trend suggests that risk factors other than the improvement in HCC diagnosis may play important roles in the development of HCC in Taiwan.

Age variation

Table 6 shows the age-specific incidence rates of liver cancer in males in six select areas.[2] Despite the fact that the age-adjusted incidence rate of liver cancer was quite similar in Khon Kaen and in Qidong, the current age curves of liver cancer incidence rates were different. The incidence rates of liver cancer for age groups < 50 years were higher in Qidong than in Khon Kaen, while the incidence rates of people ≥ 50 years of age were lower in Qidong than in Khon Kaen. This seems to suggest that the liver cancer risk factors in the two areas may be different; the exposure to risk factors occurred earlier in Qidong than in Khon Kaen or the induction period of liver cancer may be longer in Khon Kaen than in Qidong. Similar contrasts are shown in Table 6 for Bamako, Hong Kong, Osaka and Gambia with lower rates for those people below 50 years of age and higher rates for those 50 years or older in the two Asian areas than in the two African areas.

RISK FACTORS

Hepatitis viruses

Chronic infections of both hepatitis B and C viruses (HBV and HCV) are important risk factors of HCC in the world.[11,12] However, the relative risk (RR) and the population attributable risk percentage (AR%) for chronic HBV infection varied in different areas, as shown in Table 7.[13-26] The RR for the carrier status of HBV surface antigen (HBsAg) ranged significantly from 5 in Spain to more than 20 in Taiwan, Korea and Hong Kong. The AR% for HBsAg carrier status also had a wide range from less than 10% in Spain to more than 70% in Taiwan, Korea, Hong Kong and China (Table 7). The difference in RR and AR% implies that the prevalence of HCC risk factors other than HBsAg carrier status may be different in these areas. The existence of other independent risk factors may reduce the RR and AR% of HBsAg carrier status. The carrier status of HBV e antigen (HBeAg) was also associated

Table 7 Relative risk (RR) and population attributable risk percentage (AR%) of developing hepatocellular carcinoma for hepatitis B surface antigen carrier status

Country	Study design	Authors	RR	AR%
Taiwan	Cohort	Beasley *et al.*[13]	98	94
	Cohort	Chen *et al.*[14]	17	67
	Case–control	Lu *et al.*[15]	22	81
	Case–control	Chen *et al.*[16]	20	79
Korea	Case–control	Chung *et al.*[17]	41	85
Hong Kong	Case–control	Lam *et al.*[18]	21	79
China	Case–control	Yeh *et al.*[19]	17	78
Greece	Case–control	Trichopoulos *et al.*[20]	14	48
Senegal	Case–control	Prince *et al.*[21]	12	56
Philippines	Case–control	Lingao *et al.*[22]	11	64
Japan	Case–control	Tsukuma *et al.*[23]	14	21
	Cohort	Tokudome *et al.*[24]	7	11
	Cohort	Oshima *et al.*[25]	7	10
Spain	Case–control	Vall Mayans *et al.*[26]	5	8

Table 8 Relative risk (RR) and population attributable risk percentage (AR%) of developing hepatocellular carcinoma for anti-HCV seropositivity

Country	Study design	Authors	RR	AR%
Japan	Case *vs* blood donor	Watanabe *et al.*[27]	218	69
	Case–control	Tanaka *et al.*[28]	52	60
USA	Case *vs* blood donor	Hasan *et al.*[29]	134	50
	Case–control	Di Bisceglie *et al.*[30]	7	11
Spain	Case *vs* blood donor	Vargas *et al.*[31]	116	53
	Case *vs* blood donor	Bruix *et al.*[32]	38	73
South Africa	Case–control	Kew *et al.*[33]	62	29
Taiwan	Case *vs* blood donor	Chen *et al.*[34]	37	25
	Case–control	Chuang *et al.*[35]	33	17
	Case–control	Yu *et al.*[36]	24	9
Greece	Case–control	Zavitsanos *et al.*[37]	10	12
Senegal	Case–control	Coursaget *et al.*[38]	6	3
Mozambique	Case–control	Dazza *et al.*[39]	1	< 1

with an increased risk of HCC among chronic HBsAg carriers. In a recent case–control study, the RR of developing HCC was 17.9 for carriers of only HBsAg and 64.7 for carriers of both HBsAg and HBeAg compared with non-carriers as the referent.[16]

Several case series, case–control and cohort studies have shown significant associations between HCV infection and HCC risk.[27–47] The RR for the seropositivity of antibodies against HCV (anti-HCV) was much higher in case series studies than those in case–control studies, as shown in Table 8. This may be due to the choice of healthy blood donors who may have lower anti-HCV prevalence than the general population as the comparison group. The RR estimated from case–control and cohort studies was as high as 62 in South Africa and 52 in Japan, but as low as 1.1 in Mozambique (Table 8). The AR% for anti-HCV ranged from less than 1% in Mozambique to 60% in Japan (Table 8). Similarly, the wide variation in RR and AR% implies that the importance of HCC risk factors other than anti-HCV seropositivity may be different in these areas.

In areas where chronic HBV and HCV infections are both prevalent but not correlated, there may exist mutual confounding effects on HCC between HBV and HCV. The RR for chronic HBV infection will be severely underestimated if no adjustment for HCV infection is made and vice versa.[41] Furthermore, recent case–control and cohort studies have documented the synergistic effects on HCC between chronic HBV and

Table 9 Interactive effect on hepatocellular carcinoma between chronic hepatitis B and C viruses infection

Country	Study design	Authors	HBsAg/Anti-HCV seropositivity			
			–/–	–/+	+/–	+/+
Taiwan	Case–control	Yu *et al.*[36]	1.0	15.6	22.1	∞
	Case–control	Chuang *et al.*[35]	1.0	27.1	14.0	40.1
	Case–control	Tsai *et al.*[40]	1.0	92.0	29.6	96.0
	Case–control	Sun *et al.*[41]	1.0	4.0	24.6	∞
	Cohort	Chang *et al.*[42]	1.0	34.0	44.6	∞
USA	Case–control	Yu *et al.*[43]	1.0	4.8	4.4	∞
Greece	Case–control	Kalamani *et al.*[44]	1.0	11.5	4.5	74.4
Italy	Case–control	Stroffolini *et al.*[45]	1.0	21.3	13.3	77.0
Vietnam	Case–control	Cordier *et al.*[46]	1.0	36.4	76.1	*
Japan	Case–control	Tanaka *et al.*[47]	1.0	339.6	293.7	∞

HBsAg, hepatitis B surface antigen.
*Neither cases nor controls were seropositive for both HBsAg and anti-HCV.

Table 10 Selected studies on association between aflatoxin exposure and risk of hepatocellular carcinoma

Country	Study design (Aflatoxin present)	Authors	Major findings
Mozambique and South Africa	Ecological (8 districts)	Van Rensburg *et al.*[48]	$r = 0.64$*(male), $r = 0.71$*(female) (Food sampling)
Swaziland	Ecological (10 regions)	Peers *et al.*[49]	Significant association (Food sampling)
Kenya	Ecological (9 districts)	Autrup *et al.*[50]	$r = 0.75$* (Bantu people) (Urinary level)
China	Ecological (Guanxi)	Yeh *et al.*[51]	$r = 1.00$* (Food sampling)
	Ecological (49 areas)	Campbell *et al.*[52]	$r = 0.17^{\dagger}$ (Urinary level)
	Cohort (Shanghai)	Ross *et al.*[53]	RR = 3.8* (Urinary level)
Taiwan	Ecological (8 areas)	Hatch *et al.*[54]	$r = 0.29$*(male), $r = 0.17$(female) (Urinary level)
	Case–control (Penghu)	Chen *et al.*[55]	RR = 5.5* (Albumin adduct level)
	Case–control (7 areas)	Wang *et al.*[56]	RR = 5.5* (Urinary level) RR = 2.8** (Albumin adduct level)
	Cohort (Taipei)	Chen *et al.*[57]	Dose–response relation* (Albumin adduct level)

*$P < 0.05$; **$0.05 < P < 0.10$; †, not significant; RR, relative risk.

HCV infections, as shown in Table 9.[35,36,40–47] The synergistic interaction between chronic HBV and HCV infections was observed in all but one study.[40]

Aflatoxin exposure

In a cohort study, the traditional Chinese vegetarian diet has been associated with an increased risk of HCC.[14] This may result from the high consumption of fermented soy beans and their products, which may be contaminated with mycotoxins. Early case–control studies failed to find significant associations between human HCC and aflatoxin exposure.[15,16,18] This may be due to the limitation of using dietary questionnaires to assess exposure to aflatoxin. The association between aflatoxin and HCC has been documented in several ecological, case–control and cohort studies using food sampling or biomarkers to quantify aflatoxin exposure, as shown in Table 10.[48–57] Statistically significant correlations between dietary aflatoxin exposure and HCC mortality or morbidity were observed in all ecological studies performed in Mozambique,[48] South Africa,[48] Swaziland[49] and China.[51] Ecological correlation between urinary

Table 11 Selected studies on association between cigarette smoking and risk of hepatocellular carcinoma

Country	Study design	Authors	Major findings
Taiwan	Cohort	Chen *et al.*[14]	Dose–response relation*
	Case–control	Lu *et al.*[15]	Dose–response relation**
	Case–control	Chen *et al.*[16]	Dose–response relation*
	Case–control	Chen *et al.*[55]	RR = 3.6*
China	Cohort	Tu *et al.*[58]	RR = 4.6* (HBsAg carriers)
Japan	Cohort	Oshima *et al.*[25]	Dose–response relation**
	Cohort	Hirayama *et al.*[59]	Dose–response relation*
	Cohort	Goodman *et al.*[60]	RR = 2.2*
	Cohort	Kono *et al.*[61]	RR = 1.1†
	Cohort	Shibata *et al.*[62]	RR = 2.0†
	Case–control	Tanaka *et al.*[63]	Dose–response relation**
	Case–control	Tsukuma *et al.*[23]	RR = 2.0*
Hong Kong	Case–control	Lam *et al.*[18]	RR = 3.3* (HBsAg non-carriers)
USA	Case–control	Yu *et al.*[64]	Dose–response relation*
	Case–control	Yu *et al.*[65]	Dose–response relation*
	Case–control	Austin *et al.*[66]	RR = 1.0†
	Case–control	Stemhagen *et al.*[67]	RR = 0.73† (male), RR = 0.99† (female)
Greece	Case–control	Trichopoulos *et al.*[68]	Dose–response relation* (non-carriers)
	Case–control	Trichopoulos *et al.*[20]	Dose–response relation* (non-carriers)
Italy	Case–control	La Vecchia *et al.*[69]	RR = 0.9†
	Case–control	Filippazzo *et al.*[70]	No association
Sweden	Case–control	Hardell *et al.*[71]	RR = 1.4†
South Africa	Case–control	Kew *et al.*[72]	RR = 0.9†

*$P < 0.05$; **$0.05 < P < 0.10$; †, statistically non-significant; RR, relative risk.

aflatoxin levels and liver cancer mortality was statistically significant in Kenya[50] and Taiwan,[54] but not in China.[52] The cohort study performed in Shanghai has shown a significant association between urinary aflatoxin levels and HCC risk, showing a multivariate-adjusted RR of 3.8.[53] This study also showed a synergistic interaction between HBsAg carrier status and urinary aflatoxin levels. Compared with non-HBsAg carriers who had no detectable urinary level of aflatoxin as the referent (RR = 1.0), the RR of developing HCC were 1.9, 4.8 and 60.1, respectively, for HBsAg carriers with an undetectable urinary aflatoxin level, non-carriers with a detectable urinary aflatoxin level and HBsAg carriers with a detectable urinary aflatoxin level. In a recent case–control study in Taiwan, the detectable urinary aflatoxin level was also significantly associated with an increased HCC risk showing an RR of 5.5.[56] The detectable serum level of aflatoxin B_1-albumin adduct was found to be associated with an increased risk of HCC in two studies in Taiwan,[55,56] while a significant dose–response relationship between serum aflatoxin B_1-albumin adduct levels and HCC risk was observed in a cohort study performed in the Taipei metropolitan area.[57]

Cigarette smoking

The association between cigarette smoking and HCC is inconsistent in case–control and cohort studies shown in Table 11. The cigarette smoking habit was found to be associated with a significantly increased HCC risk in several studies in Taiwan,[55] China,[58] Japan,[60] and Hong Kong,[18] but not in other studies in Japan,[61,62] the USA,[66,67] Italy,[69,70] Sweden,[71] and southern Africa.[72] The dose–response relationship between cigarette smoking quantity and HCC risk has been reported in case–control or cohort studies in Taiwan,[14–16] Japan,[25,59,63] the USA,[64,65] and Greece.[20,68] The cigarette smoking effect on HCC was limited to HBsAg carriers or non-carriers in some studies, while it remained statistically significant after adjustment for HBsAg carrier status in other studies. Furthermore, a synergistic interaction between daily cigarette smoking quantity and HBsAg carrier status has also been documented.[16] Compared with HBsAg-negative non-smokers as the referent group (RR = 1.0), the RR of developing HCC were 1.8, 20.7 and 318.0, respectively, for HBsAg-negative smokers, HBsAg-positive non-smokers and HBsAg-positive smokers. There was also a synergistic interaction between cigarette smoking and anti-HCV seropositivity.[36] Compared with anti-HCV-negative non-smokers as the referent group (RR = 1.0), the RR of developing HCC were 1.6, 6.0 and 14.6, respectively, for anti-HCV-negative smokers, anti-HCV-positive non-smokers and anti-HCV-positive smokers.

Alcohol consumption

Alcohol drinking has been documented as the risk factor for HCC in most epidemiological studies shown

Table 12 Selected studies on association between alcohol consumption and risk of hepatocellular carcinoma

Country	Study design	Authors	Major findings
Taiwan	Cohort	Chen *et al.*[14]	RR = 3.1* (heavy drinkers)
	Case–control	Lu *et al.*[15]	RR = 0.6†
	Case–control	Chen *et al.*[16]	RR = 3.4* (heavy drinkers)
	Case–control	Chen *et al.*[55]	RR = 5.8* (heavy drinkers)
Japan	Cohort	Oshima *et al.*[25]	Dose–response relation*
	Cohort	Hirayama *et al.*[59]	RR = 1.9*
	Cohort	Goodman *et al.*[60]	RR = 1.2†
	Cohort	Kono *et al.*[61]	Dose–response relation*
	Cohort	Shibata *et al.*[62]	Dose–response relation*
	Case–control	Tanaka *et al.*[63]	Dose–response relation* (non-carriers)
	Case–control	Tsukuma *et al.*[23]	RR = 3.2*
Hong Kong	Case–control	Lam *et al.*[18]	No association
USA	Case–control	Yu *et al.*[64]	RR = 4.2* (heavy drinkers)
	Case–control	Austin *et al.*[66]	Dose–response relation*
	Case–control	Stemhagen *et al.*[67]	Dose–response relation*
Greece	Case–control	Trichopoulos *et al.*[68]	No association
	Case–control	Trichopoulos *et al.*[20]	No association
Italy	Case–control	La Vecchia *et al.*[69]	RR = 1.5* (heavy drinkers)
	Case–control	Filippazzo *et al.*[70]	RR = 3.2* (alcoholics)
Sweden	Case–control	Hardell *et al.*[71]	Dose–response relation*

*$P < 0.05$; †, statistically non-significant; RR, relative risk.

in Table 12. Heavy alcohol drinkers were found to have a significantly increased HCC risk showing RR ranging from 1.5 in Italy[69] to 5.8 in Penghu, Taiwan.[55] Dose–response relationship has been documented between HCC risk and alcohol consumption in both case–control and cohort studies.[25,61–63,66,67,71] But no association between alcohol consumption and HCC risk was observed in case–control studies carried out in Taiwan,[15] Japan,[60] Hong Kong,[18] and Greece.[20,68] The interaction between HBsAg carrier status and alcohol consumption was assessed in a recent case–control study in Taiwan.[16] The study found that HBsAg-negative alcohol drinkers, HBsAg-positive non-drinkers, and HBsAg-positive drinkers had relative HCC risk of 3.4, 20.2 and 75.6, respectively, compared with HBsAg-negative non-drinkers as the referent group (RR = 1.0). Another case–control study in Taiwan reported a synergistic interaction between anti-HCV seropositivity and alcohol consumption on the development of HCC.[36] Anti-HCV-negative alcohol drinkers, anti-HCV-positive non-drinkers, and anti-HCV-positive drinkers had relative HCC risk of 2.1, 6.1 and ∞, respectively, compared with anti-HCV-negative non-drinkers as the referent group (RR = 1.0).

Low vegetable consumption and serum retinol level

In a recent cohort study in Taiwan, low consumption of dark-green vegetable was associated with an increased risk of HCC.[14] Compared with those who had consumed dark-green vegetable six or more meals per week as the referent group (RR = 1), the relative risk of developing HCC was 2.6 and 4.6, for those who consumed dark-green vegetable at 2–5 and < 2 meals per week, respectively. Based on a nested case–control study of this cohort, a low level of retinol in serum samples collected at recruitment was associated with an increased HCC risk. The lower the serum retinol level, the higher the risk of HCC. The RR were 3.0 and 9.0, for those who had medium and low serum retinol levels, respectively, compared with those who had a high serum retinol level as the referent group (RR = 1.0). The reverse dose–response relationship was statistically significant among HBsAg carriers and heavy cigarette smokers, respectively, but not among non-carriers and light cigarette smokers.[73]

Inorganic arsenic ingestion

Recent ecological, case–control and cohort studies have documented a significant association between HCC and ingested inorganic arsenic through medicinal, environmental and occupational exposures in Taiwan and other countries.[74] In the endemic area of blackfoot disease, a unique peripheral vascular disease related to long-term exposure to inorganic arsenic through drinking artesian well water,[75] the age-standardized mortality ratio (SMR) of liver cancer was 170 for male and 229 for female residents compared with the general population in Taiwan as the referent (SMR = 100).[76] A dose–response relationship was reported between HCC risk and arsenic

level in drinking water in the blackfoot disease-endemic area[77] and the Taiwan Island as a whole.[78] The lifetime risk of HCC due to daily arsenic intake of 10 μg/kg was 4.3 and 3.6 per 1000, respectively, for males and females.[79] The prevalence of HBsAg carrier status (20%) among residents in the blackfoot disease-endemic area was reported to be similar to those in other areas in Taiwan.[80] The association between ingested inorganic arsenic exposure and HCC has also been documented in Japan and Germany as described in a recent review.[74] In addition to HCC, arsenic is also well documented as a carcinogen for hepatoangiosarcoma.[74]

Thorotrast, vinyl chloride and iron

Thorotrast (trade name of radioactive thorium dioxide which is used as an X-ray contrast medium) exposure is a major risk factor for the development of haemangiosarcoma and also increases the risk of cholangiocarcinoma and HCC.[81] Cohort studies in Japan[82] and Germany[83] have demonstrated a significant association between thorotrast exposure and liver cancer risk. Neither of the studies found a role of HBV infection as a cofactor. High-level exposure to vinyl chloride monomer has been well documented as a risk factor for haemangiosarcoma of the liver among polyvinyl chloride workers;[84] it is also associated with the development of cholangiosarcoma[84] and HCC.[85] No interaction between hepatitis viruses and vinyl chloride exposure has been assessed. Increased serum levels of ferritin have also been observed in liver diseases including HCC.[86] Patients affected with haemochromatosis, a genetic disease of iron overload, were found to have an increased risk of HCC.[87] The HCC risk associated with iron overload may be particularly important among patients affected with chronic HBV and HCV infection.[12]

Exogenous and endogenous hormones

Use of oral contraceptives has been well documented as a risk factor for benign hepatic adenoma; it is also associated with HCC showing a lower RR.[9,12] The HCC risk was found to increase with the duration of oral contraceptive use in developed countries with low HBV carrier rate,[88–91] but no association was observed in countries where HBV is endemic.[92,93] Hepatocellular carcinoma and cholangiosarcoma have been reported in conjunction with the use of oxymatholone, an anabolic steroid derived from testosterone.[12,94] Although a significant male-to-female ratio of HCC observed in almost all countries may be explained partly by the higher prevalence of cigarette smoking and alcohol drinking in men than in women, the elevated serum testosterone level was recently reported to be associated with an increased risk of HCC.[95] In this cohort study, elevated serum level of testosterone was associated with a relative HCC risk of 4.0 after adjustment for HBsAg carrier status, anti-HCV seropositivity, alcohol drinking, cigarette smoking, vegetable consumption frequency, past liver disease history, and vegetarian habit. However, no association between serum testosterone level and HCC was observed in China.[96]

Genetic and other diseases

Several diseases other than chronic liver diseases are associated with the development of HCC. Patients affected with haemochromatosis, porphyria and α-antitrypsin deficiency have been reported to have an increased risk of HCC.[87,97,98]

Diabetes mellitus was found to be associated with HCC showing an RR of 2.5, and drug hypersensitivity associated with an RR of 0.5.[99] There was a report of HCC related to the membranous obstruction of the inferior vena cava.[100]

Familial tendency

The familial aggregation of HCC has been well documented.[12] The familial aggregation may result from environmental factors such as HBV infection and/or genetic factors shared by family members. The RR for the family history of HCC was reported to be as high as 4.6 after adjustment for HBsAg carrier status, cigarette smoking and heavy alcohol drinking in Taiwan.[16] Segregation analyses have suggested the existence of a major autosomal gene for HCC. One study indicates the major gene is dominant,[101] but another found it to be recessive.[102] A recent segregation analysis in Taiwan also demonstrated an autosomal recessive gene for HCC.[103]

Genetic susceptibility

Human hepatocarcinogenesis is related to exposure to cigarette smoke, aflatoxins and other chemical carcinogens. Chemical carcinogens are metabolically activated by phase I enzymes including cytochrome P450 (CYP) enzymes and detoxified by phase II enzymes including epoxide hydrolase, arylamine *N*-acetyltransferase (NAT) and glutathione *S*-transferase (GST). In a nested case–control study, CYP2E1 genetic polymorphism was found to be significantly associated with the development of HCC.[104] The relative risk of developing HCC for subjects with c_1/c_1 genotype of CYP2E1 compared with those who had genotypes of c_1/c_2 or c_2/c_2 was 24.3 for cigarette smokers and 1.1 for non-smokers. Alcohol consumption was also found to increase the HCC risk among cigarette smokers with c_1/c_1 genotypes. CYP2D6-rapid metabolizers were reported to have an increased risk of HCC, while NAT2-slow acetylators are at an increased HCC risk.[105] The relative HCC risk associated with NAT2-slow and CYP2D6-rapid genotypes was 2.6 for all subjects and 5.6 for those with serum viral markers.

While no main effect on HCC was observed for the genetic polymorphism GST M1 and T1,[57,104,106] they were found to modify the associations between serum level of aflatoxin B_1 (AFB_1)-albumin adducts and HCC risk among HBsAg carriers.[57] The relative HCC risks

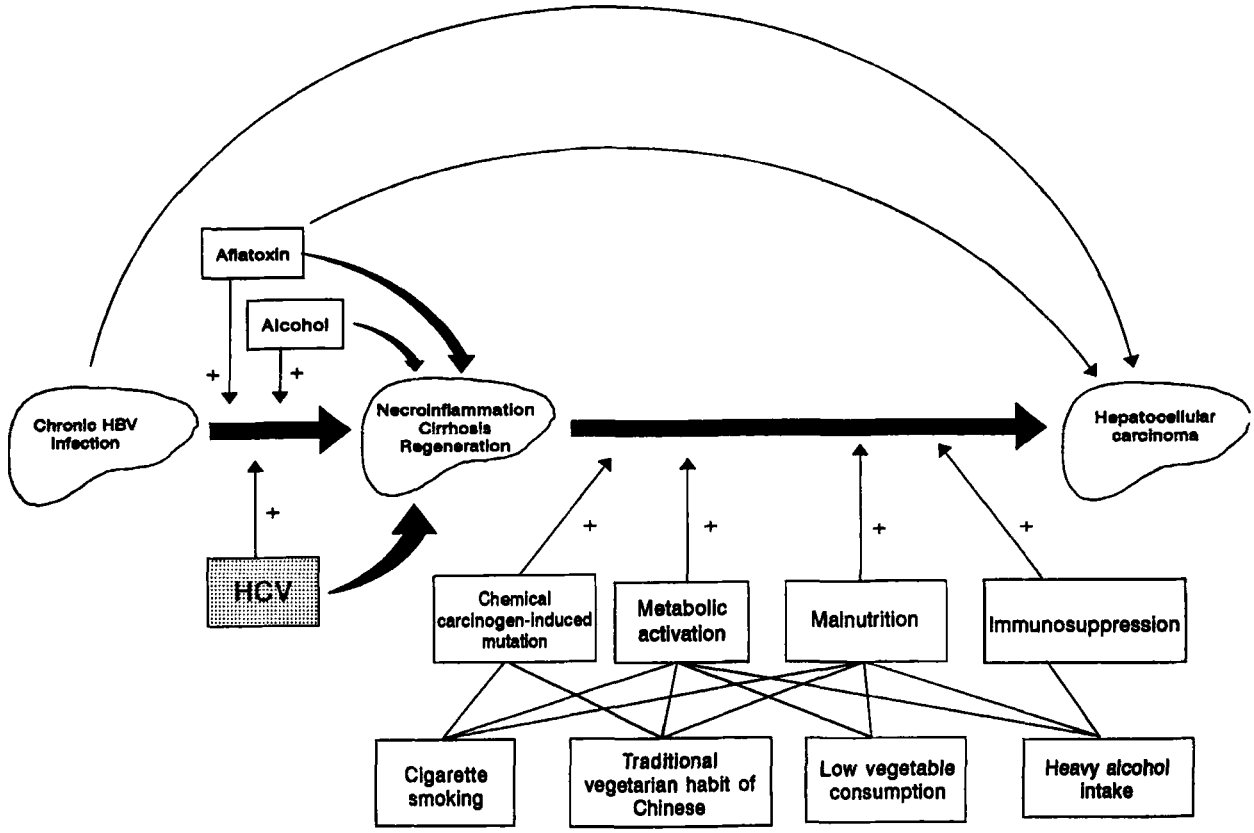

Figure 1 Hypothesized model for interactive effects of multiple risk factors for hepatocellular carcinoma in Taiwan.

were 1.6 and 3.8, respectively, for HBsAg carriers who had low and high serum level of AFB_1-albumin adducts compared with those who had undetectable adduct level as the referent group. Further stratification analysis showed the dose–response relationship was statistically significant among HBsAg carriers with null genotypes of GST M1 or T1, but not among carriers with non-null genotypes.

An increased frequency of G to T transversion in codon 249 of the *p53* tumour suppressor gene has been reported in areas where aflatoxin plays an important role in the development of HCC,[107,108] but a lower prevalence of the mutation was observed in other areas.[12] A significant association between genetic polymorphism of L-*myc* and HCC has recently been reported.[106] The RR of developing HCC for the LL genotype of L-*myc* was 2.9 compared with the L-*myc* SS genotype as the referent group. In a cohort study, the elevated serum level of *neu* oncoprotein was found as a risk predictor of HCC.[109] The higher the level of *neu* oncoprotein in serum samples collected before the diagnosis of HCC, the higher the RR of developing HCC. Furthermore, elevated serum *neu* oncoprotein level was significantly associated with HBsAg carrier status among healthy controls, and with cigarette smoking among HCC cases.

Hepatocellular carcinoma patients were reported to have a higher frequency of sister chromatid exchange (SCE) in their peripheral lymphocytes than matched controls.[110] The mean ± standard deviation of SCE was 15.1 ± 4.4 per lymphocyte at metaphase II for HCC patients and 8.9 ± 2.7 for controls who were matched with cases on age, sex, cigarette smoking and alcohol consumption. Chromosome abnormalities in peripheral lymphocytes were reported to increase among chronic HBsAg carriers.[111] Loss of chromosome arms have been recently reported in HCC tissues.[112–114]

MULTIFACTORIAL AETIOLOGY

Multistage hepatocarcinogenesis

The pathogenesis of HCC in humans is a multistage process with the involvement of multifactorial aetiology. Figure 1 shows a hypothesized model of the interaction among various risk factors for HCC in Taiwan. As reviewed by several investigators, the major pathway of hepatocarcinogenesis in Taiwan is through the HBV infection, chronic hepatitis, liver cirrhosis to HCC.[115–118] There are some HCC cases developed from chronic hepatitis directly without liver cirrhosis. HCV, aflatoxin, and alcohol may either act independently or interact with HBV to induce liver cirrhosis. In this multistage process, cigarette smoking, the traditional Chinese vegetarian habit, low vegetable consumption and heavy

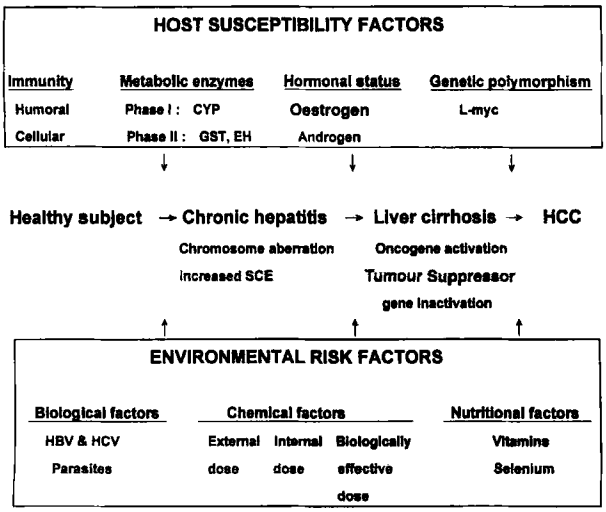

Figure 2 Host susceptibility–environment interaction and risk of hepatocellular carcinoma.

alcohol intake may increase the risk of developing HCC through their effects on the chemical carcinogen-induced mutation, metabolic activation of procarcinogens, malnutrition and/or immunosuppression. These risk factors may interact with each other synergistically to induce the development of HCC.

Different people living in different areas during different periods of time may have different sets of risk factors for HCC. In order to design strategies for the intervention or prevention of HCC, it is essential to explore these HCC risk factors for specific populations with regard to person, time and place. Only a proportion of asymptomatic HBsAg carriers are affected with chronic active or persistent hepatitis, and only some patients with chronic hepatitis will develop liver cirrhosis. The identification of other sets of risk factors which contribute to the progress from the HBsAg carrier status to chronic hepatitis and further to liver cirrhosis will assist in the prevention of HCC as early as possible. Further studies on risk factors of chronic hepatitis and liver cirrhosis are important for the effective control of HCC.

Gene–environment interaction

In addition to environmental risk factors including hepatitis viruses, liver parasites, chemical carcinogens through environmental, occupational and medicinal exposures, and dietary factors; individual susceptibility factors are also involved in the multistage development of HCC as shown in Fig. 2. These endogenous risk factors may be related to humoral and cellular immunity, metabolism of chemical carcinogens, hormonal balance and susceptibility genes. In other words, the host susceptibility may be hereditary, acquired or both. Susceptible individuals exposed to environmental risk factors tend to have the highest risk of HCC compared with those who are neither susceptible nor exposed to environmental risk factors. The elucidation of the gene–environment interactions will help the characterization of specific risk factors for specific susceptible populations. This may also improve the understanding of aetiological mechanisms of human hepatocarcinogenesis.

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