Hepatocellular carcinoma in alcoholic cirrhosis: is sex hormone imbalance a pathogenetic factor?

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Objective: A sex hormone imbalance has been reported in patients with hepatocellular carcinoma (HCC). We investigated the serum levels of eight sex hormones in patients with alcohol-related and non-alcohol-related cirrhosis and HCC.

Methods: Luteinizing hormone, follicle-stimulating hormone, estradiol, progesterone, testosterone, androstenedione, dehydroepiandrosterone and sex hormone binding globulin were assayed in 81 patients with cirrhosis (59 men, 22 women) and 97 with HCC and cirrhosis (82 men, 15 women). Hepatitis B or hepatitis C virus infection was present in 58% of patients with cirrhosis and 69% of patients with HCC. Alcohol abuse was the aetiopathogenetic factor in the remaining patients.

Results: In men, mean testosterone levels were at the lower limit of the normal range for both patients with HCC and for controls with cirrhosis. Mean estradiol levels were increased both in patients with HCC and in those with cirrhosis, but patients with alcohol-related HCC had higher estradiol levels ($P = 0.0002$). An index of sex hormone imbalance, the estradiol to testosterone ratio (ETR), was calculated. The ETR was significantly higher in patients with alcohol-related HCC ($P = 0.0002$). Multiple regression analysis showed that the ETR correlated best with patients' diagnosis ($P < 0.05$). In women, the ETR was significantly lower in patients with HCC than in controls with cirrhosis.

Conclusions: Men with alcohol-related HCC are characterized by an oestrogen and androgen imbalance and have a higher ETR than patients with other types of liver damage. Since sex hormones modulate hepatocellular proliferation, our data suggest that a sex hormone imbalance plays a role in hepatocarcinogenesis in patients with alcohol-related cirrhosis.

European Journal of Gastroenterology & Hepatology 1995, **7**:145–150

Keywords: Hepatocellular carcinoma, cirrhosis, liver neoplasm, sex hormone, ethanol, alcoholic liver disease

Introduction

Hepatocellular carcinoma (HCC) is increasing in incidence worldwide [1,2] and is invariably associated with cirrhosis [3]. Apart from cirrhosis [4], major risk factors for the development of HCC include infection with hepatitis B virus (HBV) [5,6] and, increasingly, hepatitis C virus (HCV) [7,8]. HCC, like most human cancers, is a multi-factorial, multiphasic disease in which a number of factors initiate or promote tumour development. These factors include alcohol abuse [9–11], exposure to aflatoxins [12] and possibly tobacco smoking [13]. Sex hormones may also play a role in the development of liver cancer and the long-term use of oral contraceptives has been epidemiologically linked to the development of benign [14–16] and, less clearly, malignant [17–18] liver tumours. It has been suggested that anti-oestrogens could play a role in the palliative treatment of HCC [19] and a prospective controlled study performed by our team has shown that tamoxifen can improve survival in patients with HCC [20]. However, not all HCC patients appear to respond to tamoxifen therapy and only some

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Sponsorship: Supported by the R. Farini Foundation for Gastroenterological Research.

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Date of receipt: 15 March 1994; revised: 22 August 1994; accepted: 19 October 1994.

 ISSN 0954-691X

have oestrogen receptors in neoplastic and non-neoplastic liver tissue [21,22], suggesting alternative pathways for tamoxifen activity. The aim of this study was to ascertain whether specific subsets of HCC patients have particular sex hormone imbalances which might explain their differing biological behaviours.

Patients and methods

We studied 178 patients, 97 of whom had HCC (histologically-proven by ultrasound-guided fine needle liver cytology and microhistology). The mean age of HCC patients was 60.1 years (range, 23–85 years), 82 were men and 15 women, with a male : female ratio of 5.5 : 1. The remaining 81 patients with histologically-confirmed cirrhosis but no HCC (on the basis of a negative ultrasound finding), normal alpha-fetoprotein levels [determined by radioimmunoassay (RIA), Abbott Laboratories, Chicago, Illinois, USA] and, in most cases, a 1-year follow-up formed a control group. The mean age of controls was 59.4 years (range, 26–82 years), 59 were men and 22 women, with a male : female ratio of 2.7 : 1. The age and sex of controls was not significantly different from that of the HCC patients. All female patients were menopausal.

Background information on alcohol consumption for all patients was obtained from the patients themselves and from any available family member. Alcohol abuse was defined as the consumption of more than 100 g alcohol daily in men and more than 60 g daily in women. All patients were tested for hepatitis B virus (HBV) infection markers and for anti-hepatitis C virus (HCV) antibodies (24 with first-generation and 154 with second-generation assays; Ortho Diagnostics, Tokyo, Japan). Using this approach, patients and controls were divided according to whether their disease was alcohol-related (i.e., alcohol abusers with no markers of HBV or HCV infection) or non-alcohol-related (31% of patients had alcohol-related HCC and 42% of controls had alcohol-related cirrhosis; not significant). There was an unequal distribution of alcohol-related HCC between men and women (29 out of 82 for men, one out of 15 for women; $P=0.033$ by Fischer's exact test). Thirteen and 10% of patients were hepatitis B surface antigen-positive and 56 and 48% were anti-HCV-positive in the HCC and cirrhosis groups, respectively. Patients in both groups were also divided according to their Child–Pugh status [23]: HCC Child class A (n=43), B (n=32) and C (n=22); cirrhosis Child class A (n=31), B (n=22) and C (n=28) (not significantly different).

HCC patients were also classified according to Okuda stage [24]: stage I, 60%; II, 26%; III, 14%. This was not significantly different from the Child–Pugh staging. The distribution of patients according to aetiology, sex, Child–Pugh and Okuda staging is given in Table 1.

Table 1. Patient characteristics: aetiology and staging of liver disease.

	n (%)	
	HCC (n=97)	Cirrhosis (n=18)
Aetiology		
Alcohol	30 (31)	34 (42)
HBV	13 (13)	8 (10)
HCV	54 (56)	39 (48)
Child–Pugh		
Class A	43 (44)	31 (38)
Class B	32 (33)	22 (27)
Class C	22 (23)	28 (35)
Okuda		
Okuda I	58 (60)	
Okuda II	25 (26)	
Okuda III	14 (14)	

HCC, hepatocellular carcinoma; HBV, hepatitis B virus; HCV, hepatitis C virus.

After giving informed consent, all participants provided a blood sample which was tested for the following parameters using RIA in duplicate: follicle-stimulating hormone (FSH), luteinizing hormone, estradiol, progesterone, testosterone, androstenedione, dehydroepiandrosterone (DHAS) and sex hormone binding globulin (SHBG).

An index of sex hormone imbalance was also calculated [estradiol : testosterone ratio (ETR)].

The Student's t-test, one-way analysis of variance (ANOVA) or Wilcoxon's rank sum test and Kruskall–Wallis tests were used for statistical analysis as appropriate. Multiple logistic regression analysis was also used and men and women were analysed separately. Median levels were used only for the ETR index because of the high SD for this measurement. Sensitivity, specificity, positive and negative predictive values, overall accuracy (Youden J-test) and the receiver operating capacity (ROC) curve were also determined.

Results

Men

The mean levels of the eight hormones studied in patients with HCC and in controls with cirrhosis are given in Table 2. Mean testosterone levels were at the lower limit of the normal range for both HCC patients and controls. The mean levels of androstenedione, DHAS, estradiol, progesterone and SHBG did not fall within the normal range. Androstenedione levels were higher than normal and DHAS levels were lower then normal in both HCC and cirrhosis patients; estradiol and progesterone levels were above normal in both groups, while SHBG levels were raised only in HCC patients. No statistically significant difference was observed between HCC and cirrhosis patients for any of the parameters considered.

Table 2. Sex hormones in cirrhosis and hepatocellular carcinoma (HCC) in men.

	Normal values	HCC		Cirrhosis	
		Mean	SD	Mean	SD
FSH (U/l)	1–14	6.7	8.7	7.1	9.3
LH (U/l)	1.5–9.2	6.7	6.6	6.0	7.5
T (nmol/l)	10–29	11.5	9.4	9.9	7.6
Andr (nmol/l)	0.5–1.1	2.4	2.7	3.8	4.6
DHAS (μmol/l)	5.2–8.7	0.9	0.9	1.1	1.0
E (pmol/l)	29–132	181.9	480.2	152.7	130.4
PG (nmol/l)	0–0.9	1.9	2.9	1.5	1.3
SHBG (nmol/l)	10–70	83.9	38.5	66.7	28.8

FSH, follicle-stimulating hormone; LH, luteinizing hormone; T, testosterone; Andr, androstenedione; DHAS, dehydroepiandrosterone; E, estradiol; PG, progesterone; SHBG, sex hormone binding globulin.

Estradiol and testosterone levels for patients with HCC and controls with cirrhosis grouped according to alcoholic aetiology (alcohol-related or non-alcohol-related) are given in Table 3. No differences were observed between patients with alcohol-related HCC and non-alcohol-related HCC and controls with cirrhosis with respect to testosterone, while significantly higher estradiol levels were observed in patients with alcohol-related HCC (P=0.00002 by ANOVA). No significant difference according to aetiology was observed with respect to FSH, luteinizing hormone, progesterone, androstenedione, DHEAS and SHBG.

Table 3. Estradiol and testosterone levels in men with alcohol-related and non-alcohol-related hepatocellular carcinoma (HCC) and cirrhosis.

	Mean	SD
Estradiol (pmol/l)		
HCC		
Alcoholic	311.98*	307.3
Non-alcoholic	84.98*	120.5
Cirrhosis		
Alcoholic	177.37*	156.7
Non-alcoholic	131.49*	93.7
Normal values	29–132	
Testosterone (nmol/l)		
HCC		
Alcoholic	9.91	8.9
Non-alcoholic	12.62	9.7
Cirrhosis		
Alcoholic	7.13	4.7
Non-alcoholic	12.26	8.8
Normal values	10–29	

*P=0.00002.

When patients were grouped according to aetiology and Child–Pugh score (Table 4), there was a trend towards higher estradiol levels for Child–Pugh class C patients, which reached statistical significance only for patients with non-alcohol-related HCC. HCC patients with Child–Pugh class B and C disease had significantly reduced testosterone levels, regardless of aetiology (Table 5). No significant difference between the patient groups was observed with respect to the other hormones.

Table 4. Estradiol levels in cirrhosis and hepatocellular carcinoma (HCC) by aetiology and Child–Pugh score in men.

	Alcohol-related (pmol/l)		Non-alcohol-related (pmol/l)	
	Mean	SD	Mean	SD
HCC				
Child–Pugh class				
A	126.2	101.9	61.5*	59.4
B	167.6	121.1	63.7*	74.3
C	575.3	825.6	201.6*	242.4
Cirrhosis				
Child–Pugh class				
A	100.9	56.7	101.1	61.5
B	107.1	59.9	134.7	82.8
C	221.2	264.5	156.2	131.9

Normal estradiol values, 29–132 pmol/l. *P<0.005.

Table 5. Testosterone levels in cirrhosis and hepatocellular carcinoma (HCC) by Child–Pugh class in men.

Child–Pugh class	Median testosterone levels (nmol/l)	
	Cirrhosis*	HCC**
A	10.40	16.15
B	10.80	7.63
C	4.51	3.47

*P=0.6; **P=0.00001; significance by Kruskall–Wallis.

The ETR (index of sex hormone imbalance) was higher, but not significantly, in HCC patients than in controls (20.7 versus 17.8 ETR, median values). The ETR was significantly higher in patients with alcohol-related HCC than in any other group of patients (P=0.0002) (Fig. 1). Multiple logistic regression analysis of the ETR, estradiol and testosterone levels and Child-Pugh score, showed that the ETR index had the best correlation with patients divided according to diagnosis and aetiology (P<0.05) (Table 6).

Table 6. Multiple logistic regression analysis for patient classification.

Biochemical and clinical variables	Patient classification	P
Child–Pugh	0.08473	0.08
Estradiol	–0.12110	0.21
Testosterone	0.04176	0.11
ETR	–0.16129	0.05

ETR, estradiol to testosterone ratio.

A diagnostic cut-off for the ETR with respect to alcohol-related HCC was calculated by the ROC curve

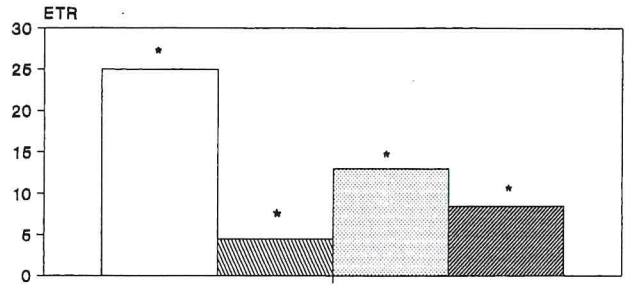

Fig. 1. Estradiol to testosterone ratio (ETR) in men with alcohol-related hepatocellular carcinoma (HCC) (□), non-alcohol-related HCC (▨), alcohol-related cirrhosis (▤) and non-alcohol-related cirrhosis (▨). Columns represent median values; *$P=0.0002$ by Kruskall–Wallis test.

(≥15 units). With this cut-off, diagnostic sensitivity was 59%, specificity 65%, positive and negative predictive values 51 and 72%, respectively, with an overall accuracy (J) of 23%. By comparison, the sensitivity of alpha-fetoprotein measurements for the same subgroup of patients was 64, 43 and 18% with cut-offs of 15, 100 and 400 nmol/l, respectively. The specificity was 100%, because a normal alpha-fetoprotein level was one of the inclusion criteria.

Women

The results obtained in female patients are shown in Table 7. Mean estradiol and testosterone levels were within the normal range in both patients with HCC and in controls with cirrhosis. DHAS levels were significantly higher in patients with alcohol-related liver damage (mean levels 1.78±1.1 versus 0.51±0.36 μmol/l; $P=0.002$).

Table 7. Sex hormones in cirrhosis and hepatocellular carcinoma (HCC) in women.

	Normal values	HCC		Cirrhosis	
		Mean	SD	Mean	SD
FSH (U/l)	35–150	47.5	32.4	35.1	32.05
LH (U/l)	11–62	40.3	46.9	13.9	12.1
T (nmol/l)	0.3–3.1	2.5	1.6	1.4	0.9
Andr (nmol/l)	1.7–10.1	0.6	0.1	0.6	0.4
DHAS (μmol/l)	0.3–1.6	0.5	0.2	1.2	1.6
E (pmol/l)	37–110	68.8	9.2	112.2	66.1
PG (nmol/l)	0.1–0.9	0.5	0.3	1.3	2.4
SHBG (nmol/l)	20–100	87.0	66.0	102.4	32.7

FSH, follicle-stimulating hormone; LH, luteinizing hormone; T, testosterone; And, androstenedione; DHAS, dehydroepiandrosterone; E, estradiol; PG; progesterone; SHBG, sex hormone binding globulin.

Only one woman had alcohol-related HCC and therefore the statistical approach used for the male patients was not applicable.

Statistically significant differences were observed in the ETR, which was lower in HCC patients than in controls with cirrhosis (29.5 versus 93.5; $P=0.036$); the levels were also significantly higher than the corresponding figures in men ($P<0.0001$ for both).

Discussion

Epidemiological, clinical and experimental data strongly suggest that the liver is involved in the metabolism of sex hormones.

The liver is a target for sex hormone activity and these hormones appear to be involved in the control of benign and malignant hepatocellular proliferative processes [14–16,19,21,25,26]. Understanding the mechanisms underlying these processes could be valuable in treating patients with HCC, as shown by our group [20] and confirmed by others [27,28].

The topic has also been addressed by three recent papers [29–31] and an oestrogen-to-androgen imbalance has been reported in HCC patients suggesting that changes in this ratio could be important in the process of liver carcinogenesis.

Effects of chronic liver damage

Our data confirm that hypogonadism is characteristic of patients with chronic liver damage, irrespective of the presence of HCC. All male patients studied had lower than normal levels of testosterone and DHAS and higher levels of androstenedione. Reduced DHAS and testosterone levels may result from increased conversion into oestrogens in the liver, skin, muscles and other tissues [32]. The raised androstenedione levels observed support the reported adrenal over-production of weak androgens, such as androstenedione, in cirrhosis [33] and imply that testicular and hepatic conversion of androstenedione into testosterone is impaired. This hypogonadism is paralleled by hyperoestrogenization: in men, about 80% of total oestrogen is produced by the liver through the aromatization of androgens into oestrogens. The liver is also involved in the final inactivation and excretion of oestrogen. In this study, estradiol and progesterone levels were higher in cirrhotic than in non-cirrhotic patients. Hyperoestrogenization is expected in cirrhosis (increased peripheral conversion of testosterone to estradiol and diminished oestrogen liver metabolism) [34], but the high levels of estradiol and progesterone in cirrhotic patients may be the result of either increased production of androstenedione and progesterone, its precursor [33], or increased production of progesterone due to alcohol abuse (present in many of our patients) because of a block at the level of 17–20 desmolase [35].

Effects of alcohol

Animal models have confirmed that alcohol abuse alone can cause hypoandrogenization with testicular atrophy [36].

Our results show that patients have a sex hormone imbalance when alcohol abuse is involved in the pathogenesis of liver damage. The ETR was higher in patients

with alcohol-related disease (both cirrhosis and HCC) than in those with non-alcoholic disease, probably because of the correlation between alcohol abuse and hypoandrogenization.

In contrast to the findings of Wang *et al.* [31], we found that patients with alcohol-related HCC had significantly higher estradiol levels and a higher ETR. The ETR in our alcoholic HCC patients was significantly higher than in any other subgroup of patients, regardless of Child–Pugh status. In particular, the difference between the ETR in patients with alcoholic cirrhosis with and those without HCC was statistically significant. This suggests that tumour development is related to the ETR or, conversely, that the ETR is modified by tumour development. In other respects, there was little difference between controls with non-alcoholic cirrhosis and patients with non-alcoholic cirrhosis and HCC. This suggests that the mechanism of tumour development related to sex hormone imbalance in alcoholic cirrhosis is different to that in non-alcoholic cirrhosis.

The ETR cannot be used to diagnose HCC in patients with alcoholic cirrhosis, but the ETR matches or surpasses the diagnostic reliability of alpha-fetoprotein measurements (depending on the cut-off used).

Sex hormone imbalance in women

Similar to patients with primary biliary cirrhosis [37], abnormal DHAS levels were found in HCC patients and were significantly higher in patients with alcohol-related liver damage. These data confirm the findings of Becker *et al.* [38].

Decreased testosterone and increased estradiol levels with a significant increase in the ETR have been described recently in female patients with alcoholic liver damage [39]. Unlike men, the ETR was significantly lower in women with HCC than in those with cirrhosis. This suggests that women with HCC have a sex hormone imbalance as severe as men with HCC but in the opposite direction. Changes in sex hormone levels may therefore play a role in the pathogenesis of liver cancer in cases of both biohumoural 'feminization' (an increased ETR) in men and of biohumoural 'masculinization' (a decreased ETR) in women.

The chronic liver regeneration that characterizes cirrhosis probably leads to cancer in most cirrhotics. Given this background, various agents may act through different mechanisms; HBV, for example, can act through integration and transactivation [40–42], aflatoxin through chemical carcinogenesis [12], HCV through still unknown mechanisms and ethanol in a variety ways [43,44]. Oestrogens modulate hepatocellular proliferation in a number of different clinical and experimental settings [14–16,21,25,26]. Alcohol-related sex hormone imbalance therefore appears to be an important co-factor in liver carcinogenesis, representing one of the possible mechanisms underlying alcohol co-carcinogenic activity. Our results as well as those by Nagasue and Guechot *et al.* [29,30], show that changes in the ETR could be used as markers of neoplastic risk in patients with alcoholic cirrhosis. Drugs such as tamoxifen (which has proved useful in inhibiting tumour proliferation in HCC patients) should be tested to ascertain whether they can inhibit neoplastic degeneration by modulating hepatocellular regeneration in the liver of cirrhotic patients at high risk, such as those with a trend towards increased alpha-fetoprotein levels.

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