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Identification of Chromosomal Aberrations by Using Trypsin G-banding in Hepatocellular Carcinoma Patients (HCC) in Tamil Nadu, India

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Abstrak: *Hepatocellular carcinoma* (HCC) (ataupun kanser hati) merupakan satu daripada malignasi manusia yang paling biasa dilaporkan di dunia. Secara etiologi, HCC berhubung kait dengan jangkitan penyakit kronik hepatitis B dan C, *cirrhosis* dan pengambilan alkohol. Objektif kajian ini ialah untuk menjelaskan perubahan kromosom (CA) dalam pesakit HCC menggunakan teknik *trypsin G-banding*. Kajian ini mungkin boleh membantu dalam pemahaman corak penyakit ini, dan untuk menilai jika perubahan kromosom ini terlibat dalam daya rentan HCC. Kajian ini telah memeriksa 51 kes HCC, dan 51 kes kawalan bebas kanser yang sama dari segi umur dan jantina, yang direkrut dari hospital-hospital di Tamil Nadu. Kes-kes HCC telah dikumpulkan mengikut umur iaitu kumpulan I (≤ 45 tahun) dan kumpulan II (≥ 46 tahun). Penghasilan penanda yang efektif untuk pengesanan HCC boleh memberi impak terhadap kesihatan awam di seluruh dunia. Subjek-subjek telah direkrut berpandukan aras serum *alpha-fetoprotein* (AFP), yang merupakan penanda efektif untuk HCC. Nombor perubahan kromatid [kumpulan I 13(25%) dan kumpulan II 43(84.3%)] dan CA [kumpulan I 10(19.6%) dan kumpulan II 28(54.9%)] yang lebih tinggi telah diperhatikan. Bagi kumpulan kawalan, keputusannya kontras, iaitu nombor perubahan kromatid [kumpulan I 5(9.8%) dan kumpulan II 12(23.5%)] dan CA [kumpulan I 4(7.8%) dan kumpulan II 9(17.6%)] ialah lebih kurang. Secara kesimpulannya, keputusan daripada kajian ini telah menyumbang terhadap pengesahan CA sebagai titik akhir pertengahan karsinogenesis. Disebabkan ramai orang tidak sedar tentang penyakit berbahaya ini, kajian ini akan membantu meningkatkan tahap kesedaran tentang kanser ini.

Kata kunci: *Hepatocellular Carcinoma* (HCC), *Alpha-fetoprotein* (AFP), Perubahan Kromosom (CA), Citogenetik

Abstract: Hepatocellular carcinoma (HCC) (or liver cancer) is one of the most common human malignancies worldwide. Aetiologically, HCC is closely associated with chronic hepatitis B and C virus infection, cirrhosis and alcohol intake. The objective of the present study was to elucidate the chromosomal abberations (CA) in HCC patients using the trypsin G-banding technique. This study may help in understanding the pattern of the disease and to assess whether these aberrations are associated with HCC susceptibility. The study examined 51 HCC cases and an equal number (*n* = 51) of age and gender matched cancer-free controls recruited from the hospitals in Tamil Nadu. The HCC cases were grouped depending upon their age into group I (≤ 45 years) and group II (≥ 46 years). The development of effective markers for the detection of HCC could have an impact on cancer mortality and may have significant public health implications worldwide. Subjects

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were recruited based on their alpha-fetoprotein (AFP) serum level, which is an effective marker for HCC. In the HCC cases, a higher number of chromatid aberrations [group I 13(25.5%) and group II 43(84.3%)] and CA [group I 10(19.6%) and group II 28(54.9%)] were observed. In contrast, controls showed a lower number of chromatid [group I 5(9.8%) and group II 12(23.5%)] and CA [group I 4(7.8%) and group II 9(17.6%)]. In conclusion, the results of this study contribute to the validation of CA as an intermediate end point in carcinogenesis. Because many people are unaware of this lethal disease, this study will raise awareness of this cancer.

Keywords: Hepatocellular Carcinoma (HCC), Alpha-fetoprotein (AFP), Chromosomal aberrations (CA), Cytogenetics

INTRODUCTION

Hepatocellular carcinoma (HCC) is one of the most common cancers in the world, accounting for 6% of all cancers worldwide particularly in countries like India, which have a high incidence of hepatitis B infection (Chakraborty *et al*. 2007; World Health Organisation 2008). Aetiologically, HCC is closely associated with chronic hepatitis B and C virus infection, cirrhosis, and aflatoxin B1 intake (Chen *et al*. 1997). Alcohol is one of the main risk factors for HCC (Stickel *et al*. 2002). Chronic hepatitis B or C, heavy alcohol use, and other conditions that damage the liver can progress over years or decades to severe fibrosis, cirrhosis, or HCC (Wong & Corley 2008). For all racial/ethnic groups, HCC rates among females have peaked at significantly lower values compared with men (Wong & Corley 2008).

An elevated level of alpha-fetoprotein (AFP) in the blood is considered the most valuable serum tumour marker for early diagnosis of HCC, which can also be monitored for recurrence after surgical resection. The development of effective markers for the detection of HCC could have an impact on cancer mortality and significant public health implications worldwide. Hence, the subjects were recruited based on their AFP serum level.

Karyotypic analysis has provided valuable information on chromosomal aberrations (CA) in a wide range of malignant diseases. Moreover, CA in peripheral blood lymphocyte culture is a hallmark of solid tumours, and it has been known for decades that chromosome rearrangements exist in most if not all human tumours (Miteiman *et al*. 1991). In HCC, deletions of chromosomal materials are common and of a non-random pattern, with recurrent deletions on chromosomes 1p, 4q, 8p, 13q, 16q, and 17p (Chan *et al*. 2002; Marchio *et al*. 1997; Piao *et al*. 1998; Wong *et al*. 2002; Nagai *et al*. 1997). The correlation between an increased frequency of CA and the hepatitis B virus (HBV) carrier state and the finding that those chronic carriers with detectable HBV in the plasma exhibited the highest frequency of chromosome breaks suggest that HBV may play a role in these genomic lesions (Nichols 1970). According to Chatterjee and Gosh (1989), metaphase chromosome spreads of peripheral blood cells of patients in the acute phase of infection with hepatitis A and B viruses exhibited an elevated level of CA and of sister chromatid exchanges.

The primary objective of the present study was to identify the extent of chromosomal damage in HCC patients in Tamil Nadu based on the clinicopathological variables of the patients. To the best of our knowledge this is the first kind of work in HCC patients in Tamil Nadu. Furthermore, the present study also aimed to identify the AFP level and it's correlation to CA formation. For this purpose, subjects were selected based on their AFP levels and further divided into group I and group II according to their age.

MATERIALS AND METHODS

Patients and Samples

Subjects for the present study were selected from patients undergoing HCC treatment in the Gastroenterology departments of various hospitals in Tamil Nadu. An ethical clearance certificate was obtained from P.S. Govindaswamy Naidu (PSG) medical hospital Coimbatore, Tamil Nadu (Ref. No: E210708-218). Fifty one subjects who had reported to the clinics were screened for CA. Equal numbers (n = 51) of mentally and physically healthy males and females were used as controls. The experimental and control subjects were grouped depending on their age into group I (≤ 45 years) and group II (≥ 46 years). The study was performed on the basis of medical reports of the practitioner (oncologist) who collected the relevant clinical details such as age, clinical and pathological data, and AFP levels. The subjects were interviewed personally and an open questionnaire requesting information on family history and other relevant details was completed by the patients. Blood samples (5.0 ml) were collected from the experimental and control subjects aseptically using heparinised tubes. The blood samples were brought to the culture laboratory in a sterile, tightly covered, ice-packed plastic container for chromosomal analysis.

Chromosomal Analysis

Karyotyping was conducted by analysing the G-banding using 5 ml of heparinised peripheral blood samples. Metaphase spreads were performed from phytohaemagglutinin (PHA) stimulated peripheral lymphocytes using standard cytogenetic techniques (Hoyos *et al*. 1996). Lymphocytes were grown in RPMI 1640 culture media (5 ml) containing antibiotics and 15% serum (1.5 ml) supplementation. PHA was added as the mitotic stimulant (0.5 ml of the inoculum) and the samples were incubated for 52 hours in a 37°C incubator. The cells were arrested at metaphase by addition of 150 µl of 0.1% colchicine.

Harvesting

The cultures were harvested after 52 hours, subsequently centrifuged at 1000 rpm for 10 minutes, and the supernatant was discarded. Hypotonic treatment was performed with KCl (5 ml) and the cultures were centrifuged a second time at 1000 rpm for 10 minutes, and the cells were fixed with 3 changes of fixative (3:1, methanol:acetic acid). Two to three fixative washes were performed until a clear suspension was obtained. The prepared slides were stained with G-bands using trypsin Giemsa stain (GTG).

Slide Preparation

The slides were cleaned overnight and subsequently washed in running tap water. Clean slides were kept in distilled water at 4°C. Three to four drops of the cell suspension were placed evenly on a chilled wet slide using a Pasteur pipette and dried on a slide warmer at 37°C. The slides were labelled and stored at room temperature.

GTG Banding

Prepared slides were treated with 0.5% trypsin solution (Sigma, USA) prepared in phosphate buffered saline (PBS) for about 5–20 seconds. The slides were rinsed thoroughly in 2 changes of PBS and stained in 10% Giemsa (Sigma, USA) solution for 10 minutes. The slides were once more rinsed in distilled water, allowed to dry and observed under the microscope. Chromosomal analysis was done under 100X magnification. Overall, 50 metaphase spreads were screened and 5 metaphases were captured using a Leica microscope.

Statistical Analysis

The statistical analyses were performed using the SPSS software for Windows, version 13. To assess the differences between HCC patients and controls, the variables are expressed as the mean ± SD and significance analysis was performed by the Mann-Whitney U test.

RESULTS

In this study a total of 102 subjects (*n* = 51 experimental subjects and *n* = 51 controls) were recruited (Table 1). In the 51 experimental subjects, 43 (84.32%) were male and 8 (15.68%) were female. Based on their age, the experimental subjects were classified into group I (≤ 45 years) and group II (≥ 46 years). The number of samples in both experimental and control subjects was 12 in group I and 39 in group II (Table 2).

All the control subjects had normal AFP level (group I 20.5 ± 6.65 and group II 19.39 ± 7.83). From the clinico-pathological data AFP levels were elevated in the experimental subjects of both group I (371.5 ± 98.90) and group II (383.64 ± 104.24). Four subjects had a family history of HCC. Eighteen of the HCC patients (35.29%) were HBV infected while alcoholic liver disease was found in 12 (23.53%) patients. Cirrhosis was observed in 40 subjects, and a tumour size < 4 cm was seen in 31 subjects (Table 2).

In control subjects, a minimal number of chromatid abberations [group I 5(9.8%) and group II 12(23.5%)] and CA [group I 4(7.8%) and group II 9(17.6%)] were detected (Table 1). In contrast, the experimental subjects showed higher numbers of chromatid aberrations [group I 13(25.5%) and group II 43(84.3%)] and CA [group I 10(19.6%) and group II 28(54.9%)] (Table 2). Experimental subjects in group II showed a higher degree of CA compared to group I. Figure 1 shows the normal karyotype whereas Figure 2 shows the abnormal karyotype of a male HCC patient.

Table 1: Frequency of chromosomal alterations in hepatocellular carcinoma (HCC) patients compared with respective controls.

S. No	Subjects		Gender	AFP level (ng/ml)		Age (yrs)		G	FH	Chromatid type aberrations		CA		Clinical and pathological data			
														E			C
	E	C		E	C	E	C	E and C	E	E	C	E	C	Cirrhosis	Etiology	Tumour size	NA
1	HCC01	CS01	M	401	26	41	40	I	–	1	–	1	1	+	HBV	<3 cm	
2	HCC02	CS02	F	421	14	53	52	II	–	2	–	1	1	+	HCV	<2 cm	
3	HCC03	CS03	M	310	12	55	65	II	–	1	1	–	–	–	HBV	<4 cm	
4	HCC04	CS04	M	213	35	45	44	I	+	2	2	–	–	+	H	<4 cm	
5	HCC05	CS05	F	355	20	71	72	II	–	1	–	–	–	+	HCV	<3 cm	
6	HCC06	CS06	M	221	13	65	58	II	–	–	–	1	–	+	ALD	<4 cm	
7	HCC07	CS07	M	193	10	55	59	II	–	–	1	–	–	+	HBV	<3 cm	
8	HCC08	CS08	M	401	09	42	42	II	–	–	–	–	–	+	HBV	<4 cm	
9	HCC09	CS09	M	410	11	44	45	I	–	1	–	1	1	–	ALD	<4 cm	
10	HCC10	CS10	M	450	20	61	60	II	–	1	1	–	–	+	U	<4 cm	
11	HCC11	CS11	M	250	15	43	42	I	–	1	1	1	1	+	HCV	<4 cm	
12	HCC12	CS12	M	512	15	47	48	II	–	–	–	1	1	–	HBV	<2 cm	

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Table1: *(continued)*

S. No	Subjects		Gender	AFP level (ng/ml)		Age (yrs)		G	FH	Chromatid type aberrations		CA		Clinical and pathological data			
														E			C
	E	C		E	C	E	C	E and C	E	E	C	E	C	Cirrhosis	Etiology	Tumour size	NA
13	HCC13	CS13	M	423	10	66	65	II	–	1	1	–	–	+	HBV / HCV	<3 cm	
14	HCC14	CS14	M	201	24	42	43	I	+	1	–	1	–	+	ALD	<4 cm	
15	HCC15	CS15	F	420	13	60	62	II	–	1	1	–	–	+	HBV / HCV	<4 cm	
16	HCC16	CS16	M	452	20	54	54	II	–	–	–	1	1	+	U	<5 cm	
17	HCC17	CS17	M	457	12	52	52	II	–	2	–	–	–	–	HBV	<3 cm	
18	HCC18	CS18	M	400	18	40	40	I	–	1	–	1	–	+	HCV	<4 cm	
19	HCC19	CS19	F	195	19	63	63	II	–	–	–	1	1	+	HBV / HCV	<4 cm	
20	HCC20	CS20	M	432	12	41	41	II	–	1	–	–	–	+	ALD	<4 cm	
21	HCC21	CS21	M	429	29	62	63	II	–	–	–	1	–	+	HBV	<2 cm	
22	HCC22	CS22	M	480	19	45	39	I	–	2	–	–	–	+	HBV	<5 cm	
23	HCC23	CS23	M	265	14	70	69	II	–	3	1	1	–	+	HBV / HCV	<4 cm	
24	HCC24	CS24	M	510	21	44	45	I	–	1	–	–	–	+	HBV	<3 cm	

(continued on next page)

Table 1: *(continued)*

S. No	Subjects		Gender	AFP level (ng/ml)		Age (yrs)		G	FH	Chromatid type aberrations		CA		Clinical and pathological data			
														E			C
	E	C		E	C	E	C	E and C	E	E	C	E	C	Cirrhosis	Etiology	Tumour size	NA
25	HCC25	CS25	M	300	20	61	62	II	–	–	–	2	1	+	U	<4 cm	
26	HCC26	CS26	M	351	15	74	72	II	–	2	–	–	–	–	HBV	<4 cm	
27	HCC27	CS27	F	421	17	76	75	II	–	1	–	2	–	+	ALD	<5 cm	
28	HCC28	CS28	M	312	18	68	69	II	–	1	2	1	–	+	HBV	<4 cm	
29	HCC29	CS29	M	402	40	55	54	II	–	1	–	1	–	+	HBV / CV	<3 cm	
30	HCC30	CS30	M	365	22	45	44	I	+	–	–	2	1	–	HBV	<4 cm	
31	HCC31	CS31	M	471	21	59	58	II	–	3	–	–	–	–	H	<4 cm	
32	HCC32	CS32	M	575	20	70	70	II	–	2	–	1	–	+	HBV	<4 cm	
33	HCC33	CS33	M	430	10	55	54	II	–	–	–	2	–	+	ALD	<2 cm	
34	HCC34	CS34	M	196	19	63	63	II	–	1	1	–	–	+	HBV / CV	<4 cm	
35	HCC35	CS35	M	366	46	53	54	II	–	1	–	2	2	+	ALD	<5 cm	
36	HCC36	CS36	M	425	18	49	48	II	–	1	–	1	–	+	HBV / HCV	<4 cm	

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Table1: *(continued)*

S. No	Subjects		Gender	AFP level (ng/ml)		Age (yrs)		G	FH	Chromatid type aberrations		CA		Clinical and pathological data			
														E			C
	E	C		E	C	E	C	E and C	E	E	C	E	C	Cirrhosis	Etiology	Tumour size	NA
37	HCC37	CS37	M	392	21	62	61	II	–	2	–	–	–	+	ALD	<3 cm	
38	HCC38	CS38	F	417	18	74	72	II	–	–	1	1	–	+	HBV / HCV	<4 cm	
39	HCC39	CS39	M	571	21	56	55	II	–	2	–	–	1	+	ALD	<4 cm	
40	HCC40	CS40	M	455	23	56	54	II	–	2	–	–	–	–	HBV	<4 cm	
41	HCC41	CS41	M	199	25	68	68	II	–	3	–	1	–	+	U	<4 cm	
42	HCC42	CS42	F	400	20	75	75	II	–	1	–	–	–	–	HBV / CV	<3 cm	
43	HCC43	CS43	M	398	33	65	64	II	–	–	2	1	–	+	ALD	<4 cm	
44	HCC44	CS44	M	435	21	63	62	II	–	2	–	1	1	+	HBV	<4 cm	
45	HCC45	CS45	F	411	20	42	41	I	+	1	1	–	–	+	U	<4 cm	
46	HCC46	CS46	M	565	18	52	52	II	–	1	–	–	–	+	ALD	<4 cm	
47	HCC47	CS47	M	399	11	44	43	I	–	1	–	1	–	+	HBV	<4 cm	
48	HCC48	CS48	M	178	26	59	57	II	–	1	–	2	–	–	ALD	<4 cm	
49	HCC49	CS49	M	418	24	44	44	I	–	1	1	2	–	+	HBV	<4 cm	

(continued on next page)

Table1: *(continued)*

S. No	Subjects		Gender	AFP level (ng/ml)		Age (yrs)		G	FH	Chromatid type aberrations		CA		Clinical and pathological data			
														E			C
	E	C		E	C	E	C	E and C	E	E	C	E	C	Cirrhosis	Etiology	Tumour size	NA
50	HCC50	CS50	M	415	10	77	77	II	–	1	–	1	–	–	H	<4 cm	
51	HCC51	CS51	M	352	22	63	63	II	–	1	–	–	–	+	HBV	<3 cm	

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Notes: **E** – Experimental; **C** – Control
M – Male; **F** – Female. Total number of subjects 51; male: 43 (84.32%); female: 8 (15.68%)
HCC – Hepatocellular carcinoma; **CS** – Control subject
AFP – Alpha-fetoprotein (in high-risk patients, AFP values between 100 and 350 ng/ml suggest a diagnosis of HCC, and levels over 350 ng/ml usually indicate the disease. Approximately 97% of the healthy subjects have AFP levels less than 8.5 ng/ml)
G – Group; **FH** – Family history
HBV – Hepatitis B virus; **HCV** – Hepatitis C virus
H – Haemochromatosis
ALD – Alcoholic liver disease
CA – Chromosomal aberrations
NA – Not applicable
\+ present
– absent
Group I and II for both the experimentals and controls shows a variation of ± 2 in age group

Table 2: General characteristics, clinico-pathological features and CAs in experimental and control subjects of the study.

S. No.	Particulars	Total number of samples	Percentage (%)
1	Total number of samples	*n* = 102	
	Experimentals	*n* = 51	100
	Controls	*n* = 51	100
	Male	*n* = 43	84.32
	Female	*n* = 8	15.68
	Group I ≤ 45 years	*n* = 12	23.53
	Group II ≥ 46 years	*n* = 39	76.47
2	AFP level (ng/ml)	Mean ± SD	
	Experimental		
	Group I	371.5 ± 98.90	NA
	Group II	383.64 ± 1.4.24	NA
	Control		
	Group I	20.5 ± 6.65	NA
	Group II	19.39 ± 7.83	NA
3	Family history (Experimental)	*n* = 4	7.8
4	Clinico-pathological features		
	i) HBV infected	*n* = 18	35.29
	ii) ALD	*n* = 12	23.53
	iii) Cirrhosis	*n* = 40	78.43
	iv) Tumor size (<4cm)	*n* = 31	60.78

Notes: **ALD** – Alcoholic liver disease
HBV – Hepatitis B virus
NA – Not applicable
n – Number of samples
AFP – Alfa-fetoprotein

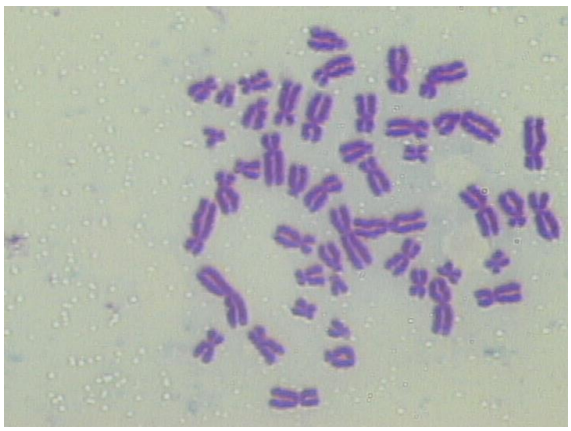

Figure 1: Karyotype showing the normal 46, XY.

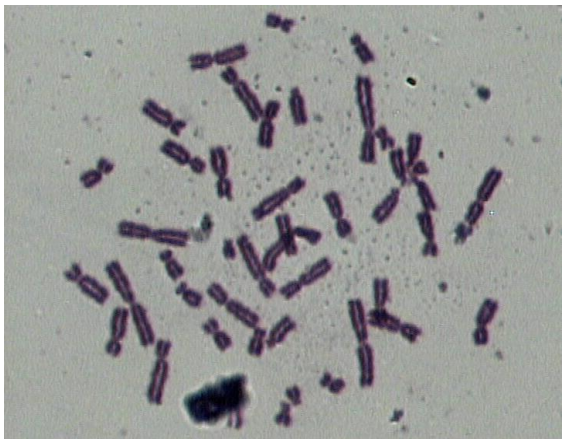

Figure 2: HCC patient (male) with abnormal karyotype.

DISCUSSION

HCC is an aggressive malignancy with a poor prognosis. Karyotyping of solid tumours yields information about numerical and/or structural chromosome defects (Balachandar *et al*. 2008b), and HCC is the most difficult carcinoma to study. Despite the available reports in literature, this is the first study to examine the spontaneous background levels of major CA in the peripheral blood lymphocyte culture (PBLC) of HCC patients in the Tamil Nadu region.

In the present study, experimental and control subjects were selected based on the AFP levels, and elevated level of AFP serum were observed in the experimental subjects of both group I (371.5 ± 98.90 ng/ml) and group II (383.64 ± 104.24 ng/ml). In contrast, the control subjects had normal AFP levels (group I 20.5 ± 6.65 and group II 19.39 ± 7.83). Approximately 70% of HCC subjects were positive for AFP, and levels of AFP increased with tumour progression (Oka *et al*. 1994). Thus, the results indicate that AFP is an effective marker for the detection of HCC in patients.

Moreover, we also sought to identify whether any relationship to age existed in HCC patients. For this purpose the experimental and control subjects were categorised into two age groups which were determined by the age at which the incidence of HCC peaked (usually between the age 50–70 years). We also separated the groups between male and female since the incidence of HCC is more common in men. In group 1 (≤ 45 years) 12 samples (23.50%) and group II (≥ 46 years) 39 samples (76.50%) were recruited. In addition, 43 (84.32%) males and 8 (15.68%) females were affected with HCC. Among males, liver cancer rates doubled every 10 years from age 40 to 60 years. In India, the mean incidence of HCC (per 100,000 population) in the 4 population-based cancer registries that were established in the nineties was 2.77 for males and 1.28 for females (Dhir & Mohandas 1998).

From the present investigation, we found that chromatid aberrations and CA were seen in both experimental and control subjects. The experimental subjects had a higher number of chromatid type aberrations in group I with 13 (25.5%) and group II with 43 (84.3%) and for CA in group I with 10 (19.6%) and group II with 28 (54.9%) (Table 1). Cytogenetically, most of the HCC patients had highly complex chromosomal changes that usually resulted in incomplete karyotypes. Cytogenetic studies of human HCC have revealed frequent abnormalities in chromosome 1q such as translocation, trisomy or amplification (Marchio *et al*. 1997; Piao *et al*. 1998; Wong *et al*. 2000) and loss of heterozygosity of chromosome region 1q42–43 and 2q35–37 in human HCC (Nagai *et al*. 1997). In the present study the major CA were deletions in 1p and 8p, translocations and satellite formations while chromatid type aberrations included gaps, breaks, and dicentrics. Chromosome 8p deletion is a recurrent CA in HCC that is commonly detected by CGH or by microsatellite analysis (Guan *et al*. 2000; Li *et al*. 2001; Wang *et al*. 2001). In HCC, deletions of chromosomal materials are common and occur in a non-random pattern, with recurrent deletions on chromosomes 1p, 4q, 8p, 13q, 16q, and 17p (Piao *et al*. 1998; Wong *et al*. 2002). In the present study, together with other clinical studies, the most commonly reported regions of interest include the 8p deletion. In one study, chromosomes 4q, 8p, and 16q were affected in HBV-related HCC, while 11q was affected in hepatitis C virus (HCV) related HCC (Wong *et al*. 1999). In the present study HBV-HCV related CA was frequently observed in 5p and 8p. Frequent allelic loss at several chromosomal regions were identified in human HCC, including 1p, 4q, 6p, 8p, 13q, 16q, and 17p (Boige *et al*. 1997; Nagai *et al*. 1997; Chen *&* Chen 1999; Jou *et al*. 2004). The high incidence of the deletion of 8p found in our study indicates that the deletion in this region is associated with the development of HCC.

We also performed pedigree analysis for both the experimental and control subjects. Interestingly four experimental subjects were associated with HCC as determined by the family based study (Table 1). Alcohol is one of the main risk factors for liver cancer (Stickel *et al*. 2002). In this study 12 of the patients were affected with alcoholic liver disease. Excessive alcohol consumption can also lead to DNA damage through the production of free radical intermediates such as reactive oxygen species, which are produced during ethanol metabolism. Cirrhosis, irrespective of cause, is a definite, established risk factor of HCC. In patients with cirrhosis, cirrhotic livers with high liver cell proliferative activity have a higher risk of developing cancer (Donato *et al*. 2001). The most likely explanation for the rising incidence of HCC is the spread of the hepatitis virus in the population. In this study most of the subjects were HBV/HCV infected in both group I and II, suggesting the possible role of HBV/HCV in the development or progression of HCC. The chromosomes that were altered in the present study were chromosome 1q, 5p, 8p, 16q, and 17p. These alterations may be due to viral infection or alcoholic cirrhosis, since most of the patients were alcohol addicts. This suggests that specific genetic abnormalities can be attributed to specific aetiological agents or mechanisms.

In controls a low number of chromatid type aberrations were seen in group I 5 (9.8%) and group II 12 (23.5%) and CA in group I 4 (7.8%) and group II 9 (17.6%) (Table 2). The results of the present study confirm that the frequency of CA in peripheral blood lymphocytes in controls is associated with age and may results from other environmental and lifestyle factors. An age-related increase in aneuploid cells in human lymphocytes has been reported in a number of studies (Carbonel *et al*. 1996; Bolognesi *et al*. 1997; Balachandar *et al*. 2008a). From the data obtained, the CAs in experimental subjects may be due to liver cirrhosis, alcohol, and hepatitis virus. As a result, the AFP levels in the blood may increase, which is an indicator for liver cancer.

CONCLUSION

The present study was conducted to reveal the genetic characteristics of liver cancer patients. This work will provide reliable information for health managers to measure the cancer status in the patients. Chromosomal abnormalities, along with life style factors, accounted significantly for the aetiology of HCC development. Compared to control patients, the HCC patients showed a high degree of chromosomal damage that made the patients susceptible to HCC. In conclusion, the results of this study contribute to the validation of CAs as an intermediate stage in carcinogenesis. In addition, AFP was shown to be an effective marker for the detection of HCC patients. It is also likely that there is a synergistic effect between HBV/HCV infection and cirrhosis in promoting HCC development. In addition to genetic factors, age, poor nutrition, lifestyle, exposure to carcinogenic pollutants, and alcohol abuse are suspected to be a cause of these cancer.

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