Hepatitis gene chip in detecting HBV DNA, HCV RNA in serum and liver tissue samples of hepatitis patients

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OBJECTIVE: To study the preparation of diagnostic gene chip for detecting hepatitis B virus (HBV) and hepatitis C virus (HCV) and its accuracy in detecting HBV DNA and HCV RNA in serum and liver tissues.

METHODS: The probes, which depend on the conservative gene fragment of hepatitis virus, was designed, synthesized and spotted on the modified glass. The probes and some other control probes were assembled on the diagnostic microarray of hepatitis virus. The gene of hepatitis virus, purified from blood or tissue, was labeled with fluorescence and hybridized to the microarray. The hybridized microarry was scanned with microarray scanner and the diagnostic result was analyzed from the scanning data. Fourty patients with hepatitis B virus and 40 healthy people or 40 patients with hepatitis C virus were subjected to detection of HBV DNA and HCV RNA with the hepatitis virus gene chip by the double-blind method. Paraffin liver specimens obtained from 99 cases of posthepatitic cirrhosis were used to detect HBV DNA. The liver tissues and serum from 15 cases of chronic hepatitis B were used to detect HBV DNA. Simultaneously, HBsAg and HBcAg were detected in the serum by fluorescence microparticle quantitation, HBV DNA and HCV RNA in the serum by PCR, and HBcAg in liver tissues by immunocytochemistry or HBV DNA by in situ molecular hybridization.

RESULTS: Chip detection of serum specimens showed that 30 patients were HBV DNA positive and 10 HBV DNA negative in the 40 patients with HBV positive, 25 patients were HCV RNA positive and 15 patients were HCV RNA negative in the 40 patients with HCV positive, and all were HBV and HCV negative in the 40 healthy people. In 15 patients with HBV marker positive who were subjected to liver biopsy, 15 patients were detected HBV DNA positive in serum by gene chip, 15 patients HBcAg positive in liver tissues by immunocytochemistry, 14 patients HBV DNA positive in liver tissues by in situ molecular hybridization, and 14 patients HBV DNA positive in liver tissues by gene chip. Paraffin liver tissues specimens from the 99 patients with posthepatitis B cirrhosis showed that 67 patients were detected HBcAg positive by immunocytochemistry, 53 patients HBV DNA positive by in situ molecular hybridization, and 46 patients HBV DNA positive by gene chip. In the 46 patients, 40 patients were detected HBV DNA and HBcAg positive by in situ molecular hybridization and immunocytochemistry, 6 patients only HBcAg positive, and 33 patients HBcAg negative.

CONCLUSIONS: The designed diagnostic gene chip can be used to simultaneously detect serum HBV DNA and HCV RNA, but the positive rate of HCV RNA diagnosed by this chip is lower. The gene chip can detect HBV DNA in serum and in liver tissue.

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Key words: hepatitis; gene chip; serum; liver tissue

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Introduction

Hepatitis gene chip is a specially designed slide on which the fluorescently-labeled probes, designed according to the characteristic gene fragment of hepatitis virus, have been arranged in the form of microarray. It can be used to make diagnosis by hybridization with nucleic acid (DNA or RNA) of hepatitis virus in the serum and liver tissue samples from patients with hepatitis, and then the chip is scanned with a given fluorescence wavelength while analyzing with computer software.[1-5] We present the results of double-blind detection of serum samples from 40 hepatitis B patients, 40 hepatitis C patients, and 40 healthy controls, and the paraffin sections of liver tissue samples from 99 patients with hepatocirrhosis, biopsy and serum samples from 15 chronic hepatitis B patients by hepatitis gene diagnostic chip, as well as the results of detection of serum markers HBsAg and HBeAg by fluorescence microparticle quantitation, serum HBV DNA and HCV RNA by PCR, HBcAg in liver tissue samples by immunohistochemistry, and HBV DNA by in situ hybridization.

Methods

Sample collection

Hepatitis B serum samples (40 patients), biopsy samples (15), and serum samples (15) were obtained from inpatients with chronic hepatitis B at Nanjing Second Hospital, Southeast University, Nanjing, China.

Serum samples of 40 healthy controls were obtained on physical examination. Paraffin sections of liver tissue samples from 99 patients with hepatocirrhosis were provided by the Department of Pathology of the hospital. Informed consent was obtained from all patients or their relatives. Serum samples were detected at the Department Laboratory Medicine of our hospital. Immunohistochemistry and in situ hybridization of liver tissue samples were performed respectively in the Department of Pathology of the same hospital to detect HBcAg and HBV DNA. Gene chip detections were performed by the Laboratory of Hepatitis in our hospital.

Reagents

The following reagents were used: super Script II reverse transcriptase (Gibco, BRL, USA), *Taq* DNA polymerase, uracil-DNA glycosidase, fluorescent primers (TaKaRa, USA).

Hepatitis diagnostic chip, and related reagents were purchased from Bohua Gene-Tech Co., Shanghai, China. Chip was prepared on slides (Sigma) and HBV DNA target gene and plant control-gene were provided by Bohua Gene-Tech Co., Shanghai, China.

PCR amplification was carried out with universal primer (25bp in length). The final concentration of dNTP was 500 μmol/L for a total reaction volume of 50 μl. The quality and concentration of PCR products were observed through 1% agarose gel electrophoresis. The products were purified through sephadex G-50 when the reaction was completed. After precipitation with propanol, the PCR products were dissolved in sample loading buffer with a concentration of 500 μg/μl for sampling using sample injector Cartesia 750. After polymerization in aqueous solution, drying, and ultraviolet cross-linking (energy value = 65 ml/cm), the products were washed with 0.2% SDS at room temperature for 5 min, rinsed twice with ddH_2O, air dried, and kept for future use.

Equipments

The following equipments were used: sample injector: Cartesian Pixsys 7500 (Cartesian, USA); scanner: ScanArry 3000 (General Scanning, USA); PCR thermal cycler: MJ Research PTC-225 (MJ Research, USA); and hybridization box: Robbins Scientific Model 1000 (Robbins Scientific, USA).

Methods

Pretreatment of serum samples (extraction)

After 250 μl reagent A was placed in a centrifuge tube, 100 μl serum was added and mixed thoroughly. Later 150 μl reagent B was added and mixed thoroughly, and again 190 μl reagent C was added and mixed thoroughly.

After centrifugation for 5 min at 13 000 rpm,

the supernatant (about 200 μl) was removed to another tube, which was added with 2.5 volume ethanol and 1/10 volume NaAc (pH 5.2), mixed thoroughly, centrifuged for 15 min at 13 000 rpm in a freezing centrifuge. After removal of ethanol, the solution was dried by air and kept for future use.

Pretreatment of liver tissue samples and DNA extraction

DNA extraction was carried out according to Goclz's method. Liver tissue samples were cut into pieces (maximal diameter <0.5 mm, 50-500 mg/10 ml), weighed, dissolved in extract solution (tissue sample <50 mg/10 ml), suspended with vortexer, laid aside for 24 hours at 48 ℃. They were suspended again, added with protease K for a final concentration of 1 mg/ml, incubated for 24 hours, centrifuged for 1 hour at 9000 g. The precipitate was washed with 70% ethanol and dried.

DNA/cDNA PCR amplification

The dried precipitate was dissolved in 10 μl deionized water, half for experiment and half for future use.

Fifteen μl reverse transcription reaction was prodenatured for 5 min in aqueous solution at 65 ℃. SuperScript II reverse transcriptase was added to the aqueous solution, which was kept at 42 ℃ for 30 min. Rtase was inactivated at 75 ℃ for 15 min. DNA/cDNA products were then taken for PCR amplification. The mixed PCR products were taken out and centrifuged for a few seconds. Twenty μl of the mixed products were taken for PCR in thermal cycler with 0.3 μl of *Taq* DNA polymerase, 0.3 μl of UNG enzyme, 5 μl of the treated sample (dissolved in 20 μl water), which had been centrifuged for a few seconds (Fig.).

Hybridization

Nine μl of the PCR products was removed to a 1.5 ml centrifuge tube. Nine μl of the hybridization solution was added and mixed thoroughly. The mixture was denatured for 5 min at 95 ℃, and put on ice immediately. Then 18 μl of the mixture was dropped to the sampling region of the dignostic chip.

Pretreatment	37 ℃		10 min		
Predenaturation	94 ℃		5 min		
		94 ℃		30 s	
		55 ℃		20 s	40 cycles
		72 ℃		20 s	
Final extension	72 ℃		5 min		
		4 ℃		forever	

Fig. DNA/cDNA PCR amplification.

After covering with a coverslip, the chip was put into a hybridization cabin and hybridized for 30 min in a hybridization box at 55 ℃. Two staining jars filled with washing solution 1 and 2 respectively were submerged in a water bath at 45 ℃. The hybridized chip was first washed in washing solution 1 for 15 min and afterwards in washing solution 2 for 10 min. The chip was then taken out and dried naturally.

Scanning

The chip was scanned on the ScanArray 3000 scanner with a scanning intensity of 55%-60%, and a correction value of -5 to obtain the CY5 portrait document. The scope of hybridization was determined by punctuating. The background noise was filtered. The fluorescence signal intensity value of gene expression was obtained. The original signal of CY5 was corrected with the previously determined control gene and the subsequently analyzed with the ImaGene 3.01 software. The criteria for positivity included: (1) the average value of the fluorescence signal of CY5 being three times as great as the value of the negative point, retro value >2.7-3.3; (2) the value of the negative fluorescence signal <800; (3) perfect PCR result.

Detection of HBcAg in liver tissue samples by using immunohistochemical SP assay

Replace experiment: physiological saline replaced the first antibody and the staining result was negative.

Negative control experiment: HBcAg in liver tissue sample from non-hepatitis B patients was stained with SP assay and the result was negative.

Positive control experiment: HBcAg in liver tissue sample from hepatitis B patients was stained with SP assay and the result was positive.

Rabbit anti-HBc antibody required for immunopathological analysis was provided by DAKO, Denmark. ABC Kit was provided by Vector, USA.

Detection of HBV DNA in liver tissue samples

The samples were digested with protease K for 15 min or with pepsin and protease K for 45 min, and rinsed with boiling water and dehydrated. After drying in an incubator at 37 ℃, the samples were added with biotin-labeled DNA probe, covered with a coverslip, heated for 10 min, laid aside in a humid box within the incubator at 37 ℃ for 5 min, swung to dry, rinsed with SP blocking solution for 5 min, visualized with DAB, rinsed with ddH_2O, restained and mounted with permount. The HBV DNA kit was provided by Vector, USA.

Detection of serum samples

Fifty-five serum samples were subjected to HBsAg and HBeAg fluorescence microparticle quantitation using Abbott's equipments and Abbott's reagents, as well as HBV DNA detection using PCR. Fourty serum samples from hepatitis C patients were detected for HCV RNA by using PCR. All of the serum samples were confirmed no cross infection.

Results

Gene chip detection of the serum samples from the 40 hepatitis B patients showed positive in 30 patients and negative in 10 patients, and that of the serum samples from the 40 hepatitis C patients

Table 1. Results of quantitative antigen assay and gene chip detection in serum samples from 30 hepatitis B patients

No.	Antigen-antibody fluorescence microparticle quantitation HBV DNA (PCR)			Fluorescence signal value of the chip		Results of gene chip detection	
	HBsAg (S/C_0)	HBeAg (PEIU/ml)	HBV DNA (PCR)	Positive reference	Average cy5	Ratio	Diagnosis
1	22.95	1959.5	+	33830	17520	2.79	+ +
2	0.54	297.0	+	30571	18281	2.85	+
3	0.51	4024.4	+	37123	3450	3.13	+
4	56.17	4078.8	+	37981	3550	3.12	+
5	14.41	3054.3	+	37622	5303	3.31	+
6	175.65	1943.3	+	31255	2007	3.11	+
7	57.92	1941.7	+	38915	24270	2.72	+
8	80.76	2593.0	+	8632	25993	3.17	+
9	2295.00	2133.9	+	23806	2486	3.25	+
10	56.17	4119.1	+	38502	2659	3.10	+
11	121.35	2300.1	+	38777	38125	3.22	+
12	57.92	3740.3	+	33030	4434	3.17	+
13	80.76	3652.4	+	40242	39430	2.28	+
14	182.35	76700.0	+	18684	28926	3.13	+
15	92.09	2644.5	+	34714	30484	3.18	+
16	7.27	2315.9	+	35709	2501	3.10	+
17	80.96	2516.1	+	29964	32608	3.93	+
18	21.95	1717.4	+	18446	1301	3.12	+
19	0.84	1716.7	+	38145	30683	2.94	+
20	56.41	1778.8	+	39564	30753	2.85	+
21	131.21	1714.6	+	33830	17520	2.79	+
22	123.21	1617.6	+	37981	3550	3.12	+
23	175.65	1679.4	+	37622	5206	2.81	+
24	57.96	1634.1	+	30084	21792	2.72	+
25	80.76	1541.9	+	86323	25993	3.17	+
26	180.36	1504.6	+	23706	2486	3.25	+
27	92.06	1331.9	+	2467	2486	3.25	+
28	7.28	1743.2	+	38502	2659	3.10	+
29	81.06	3650.9	+	38777	38124	3.22	+
30	91.06	8970.7	+	25993	4434	3.18	+

Table 2. Results of quantitative antigen-antibody assay and gene chip detection (negative) in serum samples from 10 hepatitis B patients

No.	Antigen-antibody fluorescence microparticle quantitation HBV DNA (PCR)			Fluorescence signal value of the chip		Results of gene chip detection	
	HBsAg (S/C_0)	HBeAg (PEIU/ml)	HBV DNA (PCR)	Positive reference	Average cy5	Ratio	Diagnosis
1	1.8	0.02	–	4261	40.1	0.0072	–
2	121.9	0.02	–	8182	87.6	0.0078	–
3	179.3	0.07	–	6413	98.7	0.0119	–
4	141.3	0.04	–	5142	8.2	0.0123	–
5	23.9	0.03	–	45479	231.1	0.0089	–
6	1.5	0.01	–	40959	135.2	0.0058	–
7	1.5	0.01	–	27024	389.7	0.0191	–
8	21.1	0.02	–	22764	107.2	0.0060	–
9	16.5	0.07	–	25766	109.9	0.0060	–
10	21.3	0.07	–	14976	26.2	0.0017	–

Table 3. Results of HCV PCR detection and gene chip detection (positive) in serum samples from 25 hepatitis C patients

No.	HCV RNA (PCR)	Fluorescence signal value of the chip		Results of gene chip detection	
		Positive reference	Average cy5	Ratio	Diagnosis
1	+	2882.6	2110.7	3.0	+
2	+	9164.2	4154.7	2.6	+
3	+	37033.9	4611.5	3.7	+
4	+	24872.1	4571.4	3.7	+
5	+	23710.1	2180.4	3.0	+
6	+	21452.1	2368.9	3.0	+
7	+	28910.1	1312.1	3.0	+
8	+	27653.1	2390.1	3.0	+
9	+	2613.1	2288.2	2.9	+
10	+	48170.7	2444.7	2.9	+
11	+	1498.8	2523.6	3.5	+
12	+	37157.8	2852.5	6.5	+
13	+	37033.7	1611.5	4.1	+
14	+	9164.3	1164.7	3.9	+
15	+	1743.8	3128.2	2.8	+
16	+	504.3	4535.6	2.9	+
17	+	42977.6	2561.2	3.5	+
18	+	22661.8	4241.7	3.5	+
19	+	25376.1	4148.0	4.1	+
20	+	23791.3	5170.8	2.9	+
21	+	2893.0	4321.2	2.8	+
22	+	1523.2	2184.6	3.4	+
23	+	1420.5	3157.5	4.5	+
24	+	1315.7	2145.0	4.7	+
25	+	1347.4	2167.4	4.9	+

showed positive in 25 patients and negative in 16 patients. All of the serum samples from the 40 healthy controls showed negative in gene chip detection (Tables 1-3).

Table 4. Results of quantitative antigen assay and gene chip detection in serum samples from chronic 15 hepatitis B patients

No.	Quantitation of serum antigen in hepatitis B patients		Fluorescence signal value of the chip		Results of gene chip detection	
	HBsAg	HBeAg	Positive reference	Average cy5	Ratio	Diagnosis
1	90.76	6700	3045	3237	3.7	+
2	80.76	2593	3035	3137	2.8	+
3	121.35	3654	1596	1906	6.5	+
4	92.09	2516	16008	65532	11.0	+
5	80.96	1959	59081	14090	12.0	+
6	81.06	1716	6713	8118	5.7	+
7	131.21	1778	4159	6520	7.2	+
8	175.65	1634	2183	9071	3.2	+
9	229.50	3054	3902	9338	3.7	+
10	98.50	2133	40024	41841	5.8	+
11	82.30	4119	24457	73950	5.6	+
12	82.30	4078	9717	9470	4.8	+
13	92.50	3740	9153	8714	3.6	+
14	101.01	2315	1009	1132	3.9	+
15	132.83	1679	3925	4347	5.7	+

Of the biopsy samples from the 15 patients whose serum HBV markers were positive, gene chip detection showed positive in serum of the 15 patients. As for the liver tissue samples, immunohistochemistry showed HBcAg positive in 15 patients, in situ molecular hybridization showed HBV DNA positive in 14 patients, and gene chip detection showed positive in 14 patients (Tables 4 and 5). Immunohistochemistry assay of the paraffin

Table 5. Results of the detection of HBcAg, HBV DNA and gene chip detection and light microscopy hepatohistological diagnosis in 15 chronic hepatitis B patients

No.	Light microscopy hepatohistological diagnosis	HBsAg (Immunocytotochemistry)	HBV DNA (in situ molecular hybridization)	Fluorescence signal value of the chip		Results of gene chip detection	
				Positive reference	Average cy5	Ratio	Diagnosis
1	M (G_2 S_3)	+	+	1557	5389	6.9	+
2	M (G_3 S_2)	+	+	3788	1082	3.0	+
3	M (G_4 S_4)	+	+	5472	3886	3.4	+
4	M (G_3 S_1)	+	+	9498	2760	5.1	+
5	M (G_4 S_3)	+	+	6042	8952	3.5	+
6	M (G_2 S_2)	+	+	3362	7567	7.1	+
7	M (G_3 S_3)	+	+	1517	8253	7.4	+
8	L (G_2 S_2)	+	+	3579	4250	7.5	+
9	M (G_2 S_4)	+	+	7880	9629	3.8	+
10	H (G_4 S_4)	+	+	1356	1480	3.9	+
11	L (G_3 S_1)	+	+	1894	2007	4.6	+
12	M (G_1 S_1)	+	+	3991	2089	3.7	+
13	M (G_2 S_2)	+	+	4250	4897	3.9	+
14	M (G_3 S_1)	+	+	3240	3510	3.7	+
15	M (G_4 S_3)	+	-	790	210	1.1	-

L: light; M: moderate; H: heavy; G: inflammation; S: fibrosis.

section samples from the 99 patients with liver showed HBcAg positive in 67 patients and HBcAg negative in 32 patients. HBcAg was mainly present in the cytoplasm of hepatocytes, secondly in the mesenchyma, only a few in the nuclei. The cytoplasm of hepatocytes containing HBcAg granules was diffused, brown-yellow in color and was dispersed within the liver tissue as flakes. These granules in the liver tissues were distributed diffusely in serosa type, a few in nuclear type.

In situ molecular hybridization of the 99 liver tissue samples showed HBV DNA positive in 53 patients and HBV DNA negative in 46 patients. The reaction products displayed as violet granules. Most of them were distributed diffusely in the cytoplasm and some in the nuclei of hepatocytes showing positive reaction. The positively reacted cell in the liver tissue was either dispersed as single cell or assembled as clusters. HBV DNA granules were seen in necrotic foci, capillaries, and central vein of the bile duct.

Gene chip detection of the paraffin section samples from the 99 patients with hepatocirrhosis showed positive in 46 patients, of whom, 40 were detected both HBcAg positive by immunohistochemistry and HBV DNA positive by in situ molecular hybridization. Six patients were HBcAg positive alone and 32 were HBcAg negative.

Discussion

Gene chip technique can be used to directly detect the gene fragment of a pathogen by means of molecular hybridization provided its nucleotide sequence has been already known.[6] The mothod thus is more direct and more objective than the traditional means. At present, the chip we used is a low density one made through sampling, i. e., the synthesized probes (DNA, cDNA, or oligonucleotide probes) are sampled directly on the peculiar place of the chip with a sample injector to detect hepatitis B and C, and the internal reference gene for the supervisory control system is sampled as well.

The supervisory control system consists of three main components: (1) blank point or blank sampling without any gene; (2) negative internal reference or a plant gene fragment without any homology with the HBV target gene to act as the control index of non-specific hybridization; (3) positive internal reference or adding the reference into the supervisory control system during fluorescence labeling to act as the positive internal reference of HBV. This method has many advantages: accurate quantitation, easy reproduction, lower cost, and simple manipulation. Furthermore, the design of po-

sitive and negative controls for every sample to be detected, the avoidance of contamination in PCR with molecular hybridization, and the analysis of resulting signals with computer software, the subjective function in the process of vague determination would be greatly reduced. The gene chip we used in this experiment is composed of hepatitis B positive control probes, hepatitis B detective probes and negative control probes, hepatitis C positive control probes, hepatitis C detective probes and negative control probes. All of the probes were designed on the same chip. Hence the chip can be used to detect simultaneously two types of hepatitis virus. Moreover, hybridization with the gene chip differs from PCR gene detection in the following aspects. The specific DNA hybridization with negative and positive controls, as well as the samples to be detected is carried out in the same reaction system. Obviously, it is easy to make the supervisory control analysis for the negatives and the positives in the same reaction system, thus ensuring that the false negatives could be detected by negative control and the false positives by positive control. On the contrary, in the traditional PCR detection, the supervisory control analysis for the negatives and the positives is conducted in different reaction tubes. Even in ELISA, the negative and positive controls are placed on the upper left corner and/or lower corner of the sample trough separately. Although parallel experiments on the controls and the samples could be performed, the reactions take place in different reaction tubes, and therefore the false negatives and false positives could not be avoided thoroughly.[7-9] In our experiment, serum samples from 40 healthy controls underwent double-blind detection together with the serum samples from hepatitis B and C patients. None false positive appeared.

The gene chip we designed can be used to detect HBV and HCV simultaneously. In our study, 30 serum samples which had been diagnosed hepatitis B positive by the gene chip showed high quantitative HBeAg (1331.875–8970.85 PEIU/ml) and positive in PCR HBV DNA detection. Ten serum samples that had been diagnosed hepatitis B negative by the gene chip showed negative in both HBeAg and PCR HBV DNA, and only positive in HBsAg. These results indicate that gene chip technique can be used to detect HBV DNA in serum samples properly. The positive diagnostic rate of gene chip for hepatitis C is much lower (26/40). This may be attributable to the following factors. First, the HCV RNA content in the serum of some hepatitis C patients is too low to be detected, and is degradable in the serum. Second, the amplification result of PCR is not perfect. Third, the inactivation of reverse transcriptase exerts an adverse effect on the product of reverse transcription. Fourth, the coefficient of variation in HCV is too large. Fifth, the conservation of the template is not high enough.[10-12]

We detected HBV DNA in liver tissue samples by using hepatitis B gene diagnostic chip. In comparison with the results using in situ molecular hybridization, the positive detection rate of HBV DNA was 76% (40/53). Of the 14 liver tissue samples that had been detected HBcAg positive and HBV DNA negative, 6 showed positive in gene chip detection. Of the 32 liver tissue samples that had been detected both HBcAg and HBV DNA negative, all showed negative in gene chip detection. These results indicate that there is a lower false positive rate in gene chip detection of hepatitis B. Diagnostic gene chip of hepatitis B can be used to detect HBV DNA not only in serum but also in liver tissue. The lower detection positive rate of the gene chip than that of in situ molecular hybridization may be due to the lower extraction yield of HBV DNA from the tissue samples embedded in paraffin block.[1-9] At present, formalin is commonly used as the fixative for tissue samples in most labs. The reaction of nucleic acid with formaldehyde can form the methene cross-link, thereby directly reducing the extraction yield. In the process of formalin fixation, overheating, low pH, and the existence of formic acid would break the hydrogen bonds of DNA. Moreover, the overheating and prolonged time in paraffin embedding of the tissue samples would denature DNA and produce single-stranded DNA. Single stranded DNA cannot be digested with restriction endonucleases and can result in the lower mobility in electrophoresis. Therefore, to extract the intact high-molecular-weight DNA from the tissue samples in paraffin block is the basis of a successful gene chip detection. The samples should be as fresh as possible. The best fixa-

tives are buffered formalin, acetone, and ethanol. The thick sections (1.5–20 μm) should be used and mechanical damage to DNA should be limited.[13-14] The manipulation of extraction during and after DNA release should be as gentle as possible. In order to avoid artificial DNA damage, the times of removing DNA from tube to tube should be limited. Repeated freeze-thaw should be avoided by all means so as not to make DNA degradation.

Patients with chronic hepatitis B usually have a recurrent attack. The condition of patients is usually closely related to the HBV DNA content in serum and liver tissue.[15-17] In our study, liver puncture in 15 patients with HBV DNA positive whose quantitative HBeAg range was 1634 – 4119 Eiu/ml showed high-level expression of HBcAg in the liver tissue samples by immunohistochemistry. Hence histopathological diagnosis showed active inflammation: G3 – G4 in 10 patients, G2 in 4 and G1 in 1. Twelve patients had moderately active chronic hepatitis, 2 moderate chronic hepatitis and 1 advanced chronic hepatitis.

We conclude that diagnostic chip of hepatitis gene can be used to detect HBV DNA in serum and liver tissue simultaneously. The result is fully consistent with that of in situ molecular hybridization. The success rate in extracting DNA from fresh liver tissues may be higher than that from paraffin embedded tissues. As a new detection technique, diagnostic chip of hepatitis gene can be used in clinical diagnosis of replication and expression of HBV in serum and liver tissue.

Competing interest

No benefits in any form have been received or will be received from a commercial party related directly or indirectly to the subject of this article.

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