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Study on Mutations of Resistance to Drug in Hepatitis B Virus

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This report aimed to analyze screening on mutations of resistance to drug in hepatitis B virus (HBV) during patient treatment using 1amivudine and adefovir dipivoxil. Methods **A 40-year-old Chinese man presented to Chongqing Cancer Hospital, with hepatitis B surface antigen-positive and HBeAg-positive chronic HBV and was sequentially treated with LAM 100mg/d for 16 weeks, and LAM 50 mg/d + ADV 10mg/d for 16 weeks. The primers P1(5'-AAGGGTATCTTGCCCGTTTGTCGTA-3') P2(5'-AAGCAGGATAGCCAC-AGA-3') and P3(5'-AAGGCACTTGTATTCCCATCCGAG-3') P4 (5'-AAGGTCTATTTACAG-GGGA-3') was used to analyzed the RT region of the polymerase gene from the serum of the patient at weeks 0, 18, 22, 60 and 70.** Results **The mutation M204V and T213T were detected prior to treatment, At week 18 during LAM treatment, the mutation LMV rtH69T (YMDDlocus) was identified, At week 22 during LAM treatment, the mutations LMV rtT184T(YMDDlocus) and LMV rtM204I (YMDDlocus).At week 60 during LAM + ADV treatment, the mutations ADV T213S0ADV T222A and ADV K212T were detected, at week 70 during LAM + ADV treatment, the mutations ADV S196L and ADV S242H were detected, The mutations ADV S196L and ADV S242H were detected firstly.**Conclusions **The report detected HBV mutations that escaped the antiviral pressure of LAM and LAM + ADV in the patient and provided insight into the molecular mechanism of the drug resistance for HBV virus.**

Key words: Hepatitis B virus (HBV); Resistance to Drug; Mutations; Screen.

HBV (hepatitis B virus, HBV) is a major cause of viral pathogens[1-5], caused by the HBV Hepatitis B is a serious infectious diseases endanger the health of our people. China's existing chronic hepatitis B virus infection of the world's 1/4, lamivudine (1amivudine) and adefovir dipivoxil (adefovir dipivoxil) is widely used to treat chronic hepatitis B nucleoside analogues drugs, these two drugs can be combined in a single treatment or in use in the treatment. However, due to the emergence of drug-resistant HBV strains, treatment results are often not sustainable. Some patients in the treatment or treatment of hepatitis was observed after a sudden onset, may again be detected HBV DNA, making hepatitis B treatment success rate has been low[6-11]. Clinical studies have shown, for the clinical treatment of nucleoside (acid) analogues produced HBV drug resistance is due to mutations, which led to drugs that inhibit viral replication was significantly weakened. Therefore, in the new antiviral drugs put into clinical use before resistant mutants of HBV screening, on the molecular mechanism of resistance to study and become more urgent.

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MATERIALS AND METHODS

Materials A 40-year-old male, HBsAg positive HBeAg positive, the patient not previously received antiretroviral treatment to the hospital with anti-viral nucleic acid drugs. The beginning treatment with lamivudine for four months ie 16 weeks, and then mixed with lamivudine and adefovir treatment for 16 weeks.

Determination of serum collection and related indicators were 0 weeks, 18 weeks, 22 weeks, 60 weeks and 70 weeks when collecting serum. Alanine aminotransferase (glutamate-pyruvate transaminase, ALT), and HBV DNA titers are measured using commercial kits in accordance with the determination of the clinical requirements. HBV genotype determined by direct sequencing.

Methods Serum HBV DNA extracted according to kit instructions (Omega, America), the reaction system, the total volume of 30μL, 20 mmol / L Mg2 + 3μL, 2mmol / L dNTP 3μL, 10 × buffer 3μL, primers 3μL, template 3μL, Tag enzyme 0.2 U deionized water (DH2O) make up the volume. External use a primer (P1, P2) to carry out a PCR, P1 is (5'-AAGGGTATCTTGCCCGTTTGTCGTA-3 ') P2 is (5 '-AAGCAGGATAGCCACAGA-3'), amplification conditions: 94 °C denaturation for 5min, 94 °C degeneration 30s, 55 °C annealing 30 s, 72 °C extension 60s, 36 cycles, 72 °C and then extended 10min, the first round product as a template, primers P3 (5'-AAGGCACTT GTATTCCCATCCGAG-3 ') and P4 (5'-AAGGTCTATTTACAGGGGA-3 ') for the first two PCR. Amplification conditions: 94°C denaturation for 5min, 94 °C denaturation 30s, 56 °C annealing 30s, 72 °C extension 30s, 36 cycles, 72°C and then extended 5min. Finally, the PCR products were conducted in 3% agarose gel electrophoresis, while adding the product fragments by size Marker to determine genotype.

RESULTS

HBV DNA in serum titer

Serum HBV viral titer is a reflection of how much an important indicator seen from Figure 1, the patient admitted to hospital, serum HBV DNA titer of the highest in 18 weeks, after the gradual decline in the 60 weeks minimum time, and then slightly increased.

Serum alanine transferase activity

Serum alanine transferase activity is a measure of chronic hepatitis B virus infection using the nucleotide analogue treatment outcomes important biochemical indicators seen from Figure 2, serum alanine transferase activity, the highest in at 18 weeks, gradual decline after 22 weeks remained at a lower level.

Serum HBV mutants

The patients with chronic hepatitis B virus

Table 1. Evolution of drug resistant mutatios on clonal analysis of serial samples. Values are relative of clones (%)

	Lamivydine monotherapy			Lamivydine + Adefovir dipivoxil therapy	
	0 Week	18 Week	22 Week	60 Week	70 Week
Mut ation	M204V(75.6) T213T(83.2)	LMV rtI169T YMDDlocus (58.8)			
			LMV rtT184T YMDDlocus(65.8) LMV rtM204I YMDDlocus(80.2)		
				ADV T213S(35.8) ADV T222A(55.5) ADV K212T(64.3)	
					ADV S196L(44.9, New) ADV S242H(67.4, New)

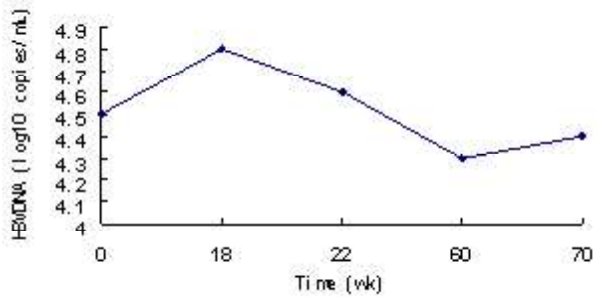

Fig. 1. HBV DNA counts

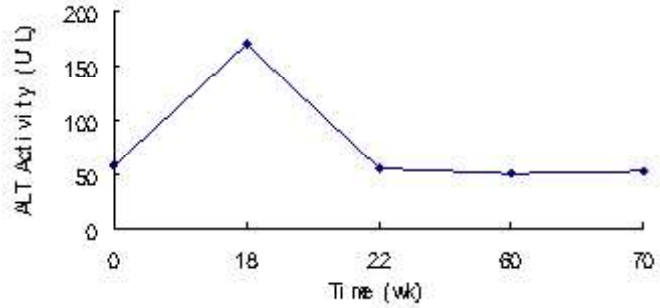

Fig. 2. Alamine aminotransferase(ALT) concentrations

infection in the use of nucleotide analogues in the treatment process, screened their serum HBV mutants. Table 1 shows that, without treatment the patient is admitted that 0 weeks, I found two mutations M204V and T213T; lamivudine therapy during the 18 weeks and 22 weeks, respectively, detect mutant LMV rtH69T (YMDDlocus) and LMV rtT184T (YMDDlocus), LMV rtM204I (YMDDlocus). In the 60 weeks and 70 weeks, respectively, detected ADV T213S, ADV T222A, ADV K212T and ADV S196L, ADV S242H, which ADV S196L and ADV S242H mutants two bell first detected.

DISCUSSION

The use of nucleoside analogues lamivudine and adefovir dipivoxil treatment of chronic hepatitis B, with the advances in treatment, HBV virus forced by environmental selection pressures are constantly mutation and evolution, which is usually said spear and shield divergence mutual relations, since the invention of nucleoside analogues lamivudine and adefovir dipivoxil since this phenomenon has been plagued by chronic hepatitis B patients, the cure rate and survival duration, clinical So far no way to get rid of this on troubled. While increasing the cost of treating patients with chronic hepatitis B and prolong the healing time.

As can be seen from this study, patients in the 0 weeks before starting treatment did not already have two mutant M204V and T213T, described as being infected patients, HBV nucleoside analogues resistant mutant virus has been in the presence of other patients may be used as the source of infection in patients receiving nucleoside analogue therapy time, HBV virus has mutated. In 18 weeks, 22 weeks and 60 weeks for patients screened mutant HBV virus, other investigators have previously been found. 70 weeks in the patients screened ADV S196L mutant HBV virus and S242H, not been discovered previously described changes in living environment condition, HBV is directed mutation of the virus, this new medicines to the treatment of chronic hepatitis B development has brought great difficulties, so troubled.

The method of detection of HBV resistance mutations, such as gene chip technology and direct sequencing of PCR products. Lamivudine and adefovir dipivoxil is

currently approved and widely used to treat chronic hepatitis B nucleoside analogue drugs, lamivudine and adefovir resistance resulting in HBV-related loci generated. Clinical studies show that the amino acid sequence of the HBV polymerase mutations, such drugs decreased affinity of HBV polymerase, that is to produce nucleoside (acid) analogues resistant to the root causes, leading to drugs that inhibit viral replication was significantly decreased . Although there are many internal and external factors, such as clinical medicine improper manner, the length of time patients aged infection gender differences affect the treatment of chronic hepatitis B, but resistant HBV mutants in patients with chronic hepatitis B is still the most important factor in treatment failure, HBV antivirals little room options to choose from, the clinical treatment of chronic hepatitis B drugs also need investment.

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