Am J Cancer Res 2017;7(6):1384-1388
www.ajcr.us /ISSN:2156-6976/ajcr0048028

Original Article

Application of AFP whole blood one-step rapid detection kit in screening for HCC in Qidong

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Received January 3, 2017; Accepted January 24, 2017; Epub June 1, 2017; Published June 15, 2017

Abstract: Hepatocellular carcinoma (HCC) is a big problem in China where the Hepatitis B (HBV) infection patients are near to 120 million. Early screening and diagnosis is the key to reduce the incidence and mortality of HCC. Serum AFP detection is the main methods for diagnosis, recurrent monitoring and therapeutic evaluation of primary HCC. Hepatitis patients should detect the AFP at least once every six months to help early diagnosis of HCC. Unfortunately, most hepatitis and other liver disease patients do not test their AFP regularly. Therefore, a rapid, convenient detect kit for AFP is necessary for the hepatitis patients to test AFP at home by themselves. It will be very helpful to the HCC early screening and early diagnosis. We screened 859 individuals who were HBsAg positive and had high risk of HCC in Qidong by using two different kits, AFP one-step rapid detection kit (Shanghai Outdo Biotech) and AFP Diagnostics ELISA kit (Zhengzhou Autobio Diagnostics), and compared the results. As a result, the positive accordance rate and the negative accordance rate of AFP one-step rapid detection kit and the Autobio ELISA kit were 95.65% (22/23) and 99.40% (831/836), respectively. The total diagnose accordance rate reached up to 99.30% (853/859). The screening results showed a high accordance rate of two methods. It is so meaningful to achieve home-test and improve HCC early screening and diagnosis by using AFP one-step rapid detection kit.

Keywords: AFP, whole blood one-step rapid detection, accordance rate, early diagnosis, HCC

Introduction

Hepatocellular carcinoma (HCC) also called malignant hepatoma, is the most common type of liver cancer. In some countries, especially in China, high incidence of hepatitis B is always along with high incidence of HCC [1]. The cure rate of early HCC can be up to 60%-70%, so experts remind that the key to HCC treatment is early detection and early treatment.

Alpha fetoprotein (AFP) is a specific tumor biomarker of HCC. It is widely used in the diagnosis of primary HCC after the first discovery. Normally, serum AFP is mainly derived from the embryo and liver cells, and it will disappear after two weeks of birth. Normally, the content of serum AFP is less than 20 μg/L. The serum AFP content is low in the healthy adult, but it will increase in most of the liver damaged patients, obviously. So far, AFP is still the best diagnostic biomarker of HCC [2]. For AFP diagnosis, if we use 20 μg/L as the detection limit, the sensitivity will be 41-65% and specificity will be 80-94%. However, if use the >200 μg/L as the detection limit, the sensitivity will be 31%, specificity will be 99% [2]. Professor Shu-seng Zheng, the academician of Chinese Academy of Engineering, has appealed that the hepatitis patients should test their AFP level at least once half a year to help early diagnosis of HCC. Similar regulations and requirements are listed in the National Prevention and Treatment Guidelines for Hepatitis and HCC.

Unfortunately, most of the patients (120 million) who have hepatitis or other liver diseases do not detect AFP levels regularly. The main reasons are including: patients do not pay enough attention to their healthy and lack of the regular

Table 1. The comparison of methods of AFP one-step test and AFP ELISA test

AFP one-step test	AFP ELISA test		
	Positive	Negative	Total
Positive	a	b	a+b
Negative	c	d	c+d
Total	a+c	b+d	N

The accordance rate was calculated as follow: Positive accordance rate=$\frac{a}{(a+c)} \times 100\%$; Negative accordance rate =$\frac{d}{(b+d)} \times 100\%$; Total accordance rate =$\frac{(a+d)}{N} \times 100\%$; For samples with inconsistent results (b and c), we used the third AFP test kit (ECL) produced by Roche Diagnostic as a verification reference.

Table 2. Correlation between AFP one-step test and AFP ELISA test

AFP one-step test	AFP ELISA test		
	Positive	Negative	Total
Positive	22	5	27
Negative	1	831	832
Total	23	836	859

inspection consciousness; the cost of detections is too high to afford; it is inconvenient when there are too many hepatitis patients get together in hospital at the same time. Therefore, once the serious symptom appears, it may be too late to cure. We need a rapid simple detection kit for early screening and detection of AFP urgently. By using the kit, hepatitis patients could detect the AFP levels at home themselves, which will greatly help the early diagnosis and early detection of HCC.

The morbidity of HCC is high in the middle and lower reaches of Yangtze River and Southeastern seaboard, especially in Qidong [3]. The incidence of HCC in Qidong which is one of the highest in China was over 50/100,000 every year in recent decades. Since 1972, the main risk factors of HCC have been fully investigated by epidemiologists, and the clinical or laboratory methods of early diagnosis and treatment were researched in Qidong [4]. After years of investigation, considering the characteristics of Qidong, we found that one of the most suitable diagnosis methods for screening high-risk group of HCC was combination of AFP and B-mode ultrasonography [5, 6].

Fund by the National "12th Five-Year" Plan for Science & Technology Support of "Large sample validation and industrialization of molecular markers for early and individualized diagnosis and treatment of hepatocellular carcinoma" (task ID 2013ZX10002007001). Shanghai Outdo Biotech Ltd carried out the development of early diagnosis of HCC, early screening, R&D and production of the grade malignancy evaluation and personalized medicine kit. And the first one-step AFP home detection product was successfully developed. It only required one drop of peripheral blood to detect the AFP expression level. Users could use the kit to monitor AFP anytime at home. In order to evaluate the practical value, we compared the AFP one-step rapid detection kit and the AFP ELISA kit by screening 859 individuals with positive HBsAg who were at-high-risk of HCC in Qidong. The results showed the sensitivity, specificity and accordance rate of AFP one-step rapid detection kit in the early detection of HCC.

Materials and methods

Samples: 859 individuals who were HBsAg positive at-high-risk of HCC in Qidong were randomly assigned by Qidong Liver Cancer Institute. There were 847 normal screening samples (No. 2638-3484), 12 abnormal retest samples.

Materials

Rapid test kit: AFP one-step rapid detection kit produced by Shanghai Outdo Biotech Co., Ltd. (20152400640).

ELISA kit: ELISA kit produced by Zhengzhou Autobio Diagnostics Co., Ltd (20153400784).

Methods

Collect the blood after fasting. Use one drop of the whole blood to test AFP by using AFP detection test kit (Shanghai Outdo Biotech Co., Ltd.), and the remaining sample was used to test AFP after separation of plasma by using AFP ELISA kit. Follow the kits instructions to conduct test completely.

As a qualitative method, the LODof AFP rapid test kit was 20 μg/L. The results of AFP tested by ELISA kit were classified as positive if the AFP concentration ≥20 μg/L, while those with less than 20 μg/L AFP were classified as negative. The analysis method of accordance rate between AFP rapid test kit and AFP ELISA kit were shown in **Table 1**.

Table 3. The detection results of AFP one-step kit in different AFP concentration range

AFP concentration (μg/L)	AFP ELISA test	AFP one-step test
<10	827	2 cases positive, 825 cases negative
10-19	9	3 cases positive, 6 cases negative
20-49	4	3 cases positive, 1 cases negative
50-199	11	11 cases positive*
200-400	4	4 cases positive
>400	4	4 cases positive*

*Note: Two patients were found having placeholder. One patient's AFP was more than 400 μg/L, and the others' AFP concentration was about 50-100 μg/L.

Table 4. Retest results of inconsistent samples

	No.	AFP one-step test	AFP ELISA test	AFP ECL test
1	3048*	+	<10	10.96
2	3057*	+	10-20	16.58
3	3135	+	10-20	37.83
4	3148*	+	<10	14.88
5	3381	+	10-20	2.41
6	Retest 2	-	20-50	1.71

*Note: Three samples that detected by AFP one-step test using whole blood was weakly positive, but negative using plasma, were all classified into negative in analysis.

Results

Accordance rate

Take the AFP ELISA result as a standard. The screening results are as following: there were 27 positive samples of AFP one-step test, while 23 of AFP ELISA test and 22 samples showed same positive results by two different methods. There were 832 negative samples of AFP one-step test, while 836 of AFP ELISA test. 831 samples showed same negative results by two different methods (**Table 2**).

The accordance rate was calculated as following:

Positive accordance rate = 22/23×100% = 95.65%

Negative accordance rate = 831/836×100% = 99.40%

Total accordance rate = (22+831) /859×100% = 99.30%

Different concentration of AFP

Based on the results of AFP ELISA quantitative, the concentration of AFP in screening of high-risk HCC in Qidong is as following: 827 cases were less than 10 μg/L, 9 cases 10-19 μg/L, 4 cases 20-49 μg/L, 11 cases 50-199 μg/L, 4 cases 200-400 μg/L, 4 cases higher than 400 μg/L. The results of AFP rapid detection kit in different concentration ranges are shown in **Table 3**.

Inconsistent results samples

A total of 6 cases had different results by two different methods during the screening process. 5 out of 6 were detected positive by AFP one-step rapid detection kit but negative by AFP ELISA kit (No. 3048, 3057, 3135, 3148, and 3381). 1 sample is negative by AFP one-step rapid kit detection but positive by AFP ELISA test (No. retest 2). The 6 samples were retest by the third testing kits-Roche AFP ECL detection kit for verification. Specific results are shown in **Table 4**.

Based on the results of AFP ECL and AFP ELISA, 1 out of 6 retest samples was AFP ELISA missed (No. 3135, true positive by AFP one-step test), and 1 case was AFP ELISA detection error (retest 2, true negative by AFP one-step test). The other 4 retest samples were AFP one-step detection error (No. 3048, 3057, 3148, 3382, false positive by AFP one-step test).

Discussion

As a national "Tumor Registration Demonstration Base" and "HCC Early Detection and Treatment Demonstration Base", Qidong Liver Cancer Institute provided valuable biological basis data and experimental evidence for the research of epidemiology, etiology, early detection and treatment, and biological markers of HCC in recent 40 years, which have a great impact in China and international cooperation [7-11]. Based on this, Shanghai Outdo Biotech Co., Ltd in cooperation with Qidong Liver Cancer Institute applied AFP one-step rapid detection kit in "HCC early detection and treatment demonstration project" to evaluate the sensitivity and specificity of the kit.

In this study, we screened 859 patients (including 847 normal samples, 12 abnormal sam-

ples) in the Qidong area. The result was compared with the ELISA kit indicating that the positive and negative accordance rate between AFP one-step rapid detection kit and the ELISA kit were 95.65% (22/23) and 99.40% (831/836), respectively. The total diagnose accordance rate reached up to 99.30% (853/859). The screening results proved a high accordance rate of two methods.

For the 6 samples with inconsistent results retested by AFP ECL (Roche Diagnostic), 1 sample with 20-50 μg/L AFP was missed and 1 sample was wrong detected by ELISA kit. The other 4 retest samples were wrong detected by AFP one-step rapid kit, including 3 samples of AFP 10-19 μg/L concentration on gray area. We analysis the results of two kits in different AFP concentration range, and found out that inconsistent results appeared mainly in mildly elevated AFP (20-49 μg/L) and detection gray areas (10-19 μg/L). For high concentration AFP samples (AFP concentration above 50 μg/L), the diagnose accordance rate of two test kits was 100%; while the diagnose accordance rate of the majority of AFP normal group (AFP concentration of less than 10 μg/L) was up to 99.76%. Based on these results, we confirmed that there was no significant difference in the detection capability between AFP one-step rapid kit and AFP ELISA kit.

The results of this study showed the excellent characteristics of the AFP one-step rapid detection kit, including sensitivity, specificity, quick interpretation of the results, simple without additional equipment, and only need a drop of whole blood. It is a very suitable product for early detection and diagnosis of HCC in large-scale screening.

During the screening process, there were two patients who were detected positive by AFP rapid detection kit, and liver lesions were found by B-mode ultrasonography. One patient's AFP was 400 μg/L, another's AFP was about 50-100 μg/L and the B-mode ultrasonography results showed that liver lesions were small. Both of them were diagnosed as early HCC. The screening result indicated the AFP one-step rapid detection kit playing a very important role in screening and diagnosis of early HCC.

China is one of the biggest countries of high incidence of HCC and hepatitis B or hepatitis C carries, thus HCC early detection and treatment is quite essential. However, restricted by the limited medical resources, "centralized" early detection and treatment for HCC is not fully rolled out. Therefore, early HCC screening through low-cost, independent of time and place, suitable for everyone in the "family" would be an effective method to solve the above problems. As the first AFP "finger stick" one-step home-test product in China, AFP one-step rapid detection kit with features of convenient, sensitive, rapid and accurate has been well-proved in practical use. Using the kit, the hepatitis patients could detect their AFP at home by themselves and monitor their AFP levels at anytime. When positive result is found, which indicates the AFP level has increased, the patients could realize they must go to the hospital for further inspection. Thus, a huge number of early HCC or other liver diseases will be diagnosed in time. The AFP one-step rapid kit will greatly benefit the 120 million patients suffered from hepatitis and other liver diseases. It has far-reaching significance for promoting the further development of the technical proposal in early diagnosis and early treatment of HCC and the future practice at home-test of HCC early monitoring or screening.

Disclosure of conflict of interest

None.

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