DETERMINATION OF ALPHAFETOPROTEIN (AFP) LEVEL IN THE HEPATITIS B CARRIERS WITH DIFFERENT MALIGNANT DISEASES BY RADIOIMMUNOASSAY TECHNIQUE (A PILOT STUDY)

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ABSTRACT

Alpha-fetoprotein (AFP) , is a commonly used tumor marker in the present day. In the Individuals with chronic hepatitis B are at increased risk for the development of hepatocellular carcinoma. Although precise recommendations do not exist, it is reasonable for such individuals to undergo periodic screening for cancer. Screening procedures include measurement of serum alpha-fetoprotein (a tumor marker that is elevated in about 85% of individuals with hepatocellular carcinoma) and ultrasound examination. Here we reported the study of serum AFP levels determined by radioimmunoassay technique in the hepatitis B carriers with various malignant diseases. This study was designed as a descriptive pilot study. The serum AFP levels from 7 hepatitis B carriers with different malignant diseases, 3 with hepatoma (Child Class C) and 4 with non-hepatoma, were used in this study. All were the confirmed cases of hepatitis B infection without concomitant of other hepatitis. The radioimmunoassay kit used in this study was ELISA-AFP (CIS Bio International). It was noted that a great variations of the AFP levels among the hepatoma subjects were observed although we used the patient in the same Child classification. However, comparing to the other 10 selected non hepatitis B carriers with hepatoma (Child Class C), wide range of serum AFP was also observed. Furthermore, some overlapping of the AFP levels between hepatoma and non-hepatoma group was observed. We also studied the serum AFP levels among the other 10 hepatitis B silent carriers without evidence of liver structural pathology (from ultrasonography study) and revealed that the average AFP level was comparable to the malignant group. Furthermore, using the ANOVA analysis, there was no significant difference among the four groups of subjects ($P = 0.176$). Hence, the serum AFP level may not be a good screening tool for hepatoma in the hepatitis B carriers.

Key words: alpha fetoprotein, hepatitis B carrier, radioimmunoassay

INTRODUCTION

Alpha-fetoprotein (AFP) is a glycoprotein with a molecular weight of approximately 70 KD.[1] AFP is normally produced during fetal and neonatal development by the liver, yolk sac, and in small concentrations by the gastrointestinal tract.[2] After birth, serum AFP concentrations decrease

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rapidly, and by the second year of life and thereafter only trace amounts are normally detected in serum.[3] Elevation of serum AFP to abnormally high values occurs in several malignant diseases,[4-7] most notably nonseminomatous testicular cancer and primary hepatocellular carcinoma. In the case of nonseminomatous testicular cancer, a direct relationship has been observed between the incidence of elevated AFP levels and the stage of disease.[8-9]

Elevated AFP levels have also been observed in patients diagnosed with seminoma with nonseminomatous elements, but not in patients with pure seminoma.[6,8,10,11] In addition, elevated serum AFP concentrations have been measured in patients with other noncancerous diseases, including ataxia telangiectasia, hereditary tyrosinemia, neonatal hyperbilirubinemia, acute viral hepatitis, chronic active hepatitis and cirrhosis.[12-15] Elevated serum AFP concentrations are also observed in pregnant women.[16-17] Therefore, AFP measurements are not recommended for use as a screening procedure to detect the presence of cancer in the general population.

However, in the Individuals with chronic hepatitis B are at increased risk for the development of hepatocellular carcinoma. Although precise recommendations do not exist, it is reasonable for such individuals to undergo periodic screening for cancer. Screening procedures include measurement of serum alpha-fetoprotein (a tumor marker that is elevated in about 85% of individuals with hepatocellular carcinoma) and ultrasound examination. Here we reported the study of serum AFP levels determined by radioimmunassay technique in the hepatitis B carriers with various malignant diseases.

MATERIALS AND METHODS

This study was designed as descriptive pilot study. The serum AFP levels from 7 hepatitis B carriers with different malignant diseases, 3 with hepatoma (Child Class C) and 4 with non-hepatoma, were used in this study. All were the confirmed cases of hepatitis B infection without concomitant of other hepatitis. The radioimmunoassay kit used in this study was ELISA-AFP, purchased from the CIS Bio International. Briefly, it is a solid phase two-sited immunoturbidimeteric assay. Two monoclonal antibodies were prepared against sterically remoted antigenic sites on the AFP molecules, the first one is coated in the ELISA solid phase, the second one radiolabelled with iodine 125 is used as a tracer. The AFP molecules present in the standards or the samples to be tested are "sandwiched" between the two antibodies, following the formation or the coated antibodies/antigen/iodinated antibody sandwich, the unbound tracer is easily removed by a washing step.

All collected data were analyzed using descriptive statistical analysis. Comparison was performed at the statistical significant level equaled to 0.05.

RESULTS

The result from AFP determination was shown in Table 1. The AFP levels among the hepatoma group ranged from 3 to 800 ng/mL while the AFP levels among the non-hepatoma group ranged from 4 to 9.5 ng/mL (Figure 1).

Table 1. Serum AFP levels among the hepatitis B carrier subjects

No	Disease	Serum AFP level (ng/ml)
1	Hepatoma, Child Class C	800
2	Hepatoma, Child Class C	8
3	Hepatoma, Child Class C	3
4	Non Hodgkin's lymphoma	6.5
5	CA esophagus	9.5
6	CA cervix, stage III B	4.5
7	Cholangiocarcinoma	4

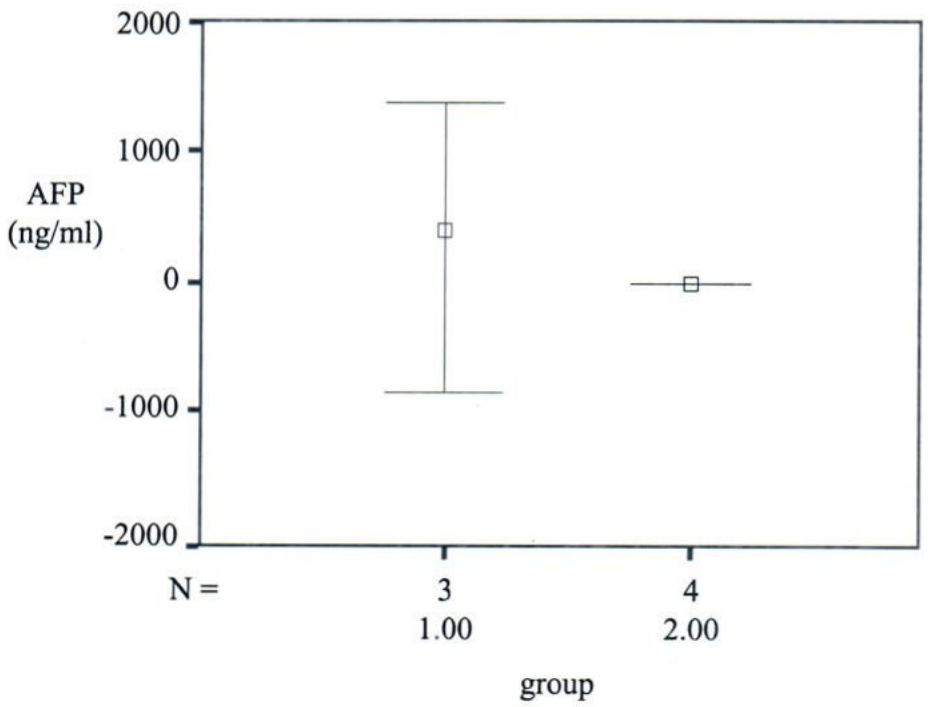

Fig. 1. Show 95% CI of serum AFP levels in each group
Group 1 = hepatitis B carriers with hepatoma
Group 2 = hepatitis B carriers with non hepatoma malignant diseases

Table 2. Average (mean ± SD) and 95 % CI of serum AFP level in different groups of patients

Group	Average (ng/ml)	95 % CI (ng/ml)
1. hepatitis B carriers with hepatoma (n = 3)	270.33 ± 458.71	0 - 1409.84
2. hepatitis B carriers with non hepatoma malignant diseases (n = 4)	6.13 ± 2.50	2.15 - 10.10
3. silent hepatitis B carrier (n = 10)	5.50 ± 1.58	4.37 - 6.63
4. non hepatitis B carriers with hepatoma (n = 10)	146.40 ± 182.69	15.71 - 277.09

DISCUSSION

HBV is a mostly double-stranded DNA virus in the Hepadnaviridae family. HBV causes hepatitis in human and related virus in this family cause hepatitis in ducks, ground squirrels and woodchucks. Although relatively rare in the United States, hepatitis B is endemic in parts of Asia where hundreds of millions of individuals may be infected. HBV is transmitted horizontally by blood and blood products and sexual transmission. It is also transmitted vertically from mother to infant in the perinatal period which is a major mode of transmission in regions where hepatitis B is endemic.[18-20]

HBV causes acute and chronic hepatitis. The chances of becoming chronically infected depends upon age. About 90% of infected neonates and 50% of infected young children will become chronically infected. In contrast, only about 5% to 10% of immunocompetent adults infected with HBV develop chronic hepatitis B.[18-20] These patients, who are still potentially infectious, have no symptoms and no abnormalities on laboratory testing. Nonetheless, some of these patients will have evidence of hepatitis on liver biopsy. Some individuals with chronic hepatitis B will have clinically insignificant or minimal liver disease and never develop complications. Others will have clinically apparent chronic hepatitis. Some will go on to develop cirrhosis. Individuals with chronic hepatitis B, especially those with cirrhosis but even so-called chronic carriers, are at an increased risk of developing hepatocellular carcinoma (primary liver cancer). This type of cancer is the leading cause of cancer death in the world, also in Thailand.

Therefore, every carrier needs a yearly check-up with the doctor to find the possible complications. A panel of liver enzymes (e.g., AST, alkaline phosphatase), liver function tests (e.g., PT) and a complete blood count should be checked yearly.[21] A baseline AFP and ultrasound are usually obtained at the first clinical visit, no matter what the patient's age is. In cirrhosis, yearly or twice yearly testing is begun as early indicators of hepatocellular carcinoma (HCC). The AFP is a tumor marker and is increased in 85% of individuals with HCC, often before clinical evidence of cancer is present. Ultrasound may show small cancers at a time when they can be resected completely.

Here, we performed a pilot study to obtain the level of serum AFP in hepatitis B carriers from different malignant diseases. Interestingly, the great variations of the AFP levels among the hepatoma subjects were observed although we used the patient in the same Child classification. However, comparing to the other

10 selected non hepatitis B carriers with hepatoma (Child Class C), wide range of serum AFP was also observed (Table 2). Furthermore, some overlapping of the AFP levels between hepatoma and non-hepatoma group was observed.

We also studied the serum ALP levels among the other 10 hepatitis B silent carriers without evidence of liver structural pathology (from ultrasonography study) and revealed that the average AFP level was comparable to the malignant group (Table 2). Furthermore, using the ANOVA analysis, there was no significant difference among the four groups of subjects ($P = 0.176$). Hence, the serum AFP level may not be a good screening tool for hepatoma in the hepatitis B carriers. However, our study is only a pilot study with only a few subjects, therefore, further larger study is recommended.

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