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Serum alpha-fetoprotein levels in healthy Japanese adults.

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Abstract

With advances in lectin affinity electrophoresis of alpha-fetoprotein (AFP), the detection of significant changes in serum AFP at low levels in cirrhotics has become important for early detection of hepatocellular carcinoma. Serum AFP levels of 616 healthy individuals without abnormal liver function tests or virus markers of hepatitis B and C were determined by enzyme immunoassay with IMx-AFP Dainapack using automated IMx apparatus set at twice the ordinary sensitivity and compared with those of 241 individuals with abnormal liver function tests and/or positive hepatitis virus markers. The coefficient of variation in this assay was less than 10% at AFP levels as low as 0.2 ng/ml with a lower detection limit of 0.1 ng/ml. The AFP level of healthy population showed a Gaussian distribution curve after logarithmic transformation with a median and 2.5-97.5 percentile reference range of 2.2 (0.6-5.6) ng/ml. There was no significant difference in the AFP level between males and females. Individuals with abnormal liver function tests alone showed no significant increase in serum AFP unless they were associated with positive hepatitis virus markers.

KEYWORDS: ?-fetoprotein, enzyme immunoassay, healthy japanese adults, serum level, reference values

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Serum α-Fetoprotein Levels in Healthy Japanese Adults

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With advances in lectin affinity electrophoresis of α-fetoprotein (AFP), the detection of significant changes in serum AFP at low levels in cirrhotics has become important for early detection of hepatocellular carcinoma. Serum AFP levels of 616 healthy individuals without abnormal liver function tests or virus markers of hepatitis B and C were determined by enzyme immunoassay with IMx-AFP Dainapack using automated IMx apparatus set at twice the ordinary sensitivity and compared with those of 241 individuals with abnormal liver function tests and/or positive hepatitis virus markers. The coefficient of variation in this assay was less than 10% at AFP levels as low as 0.2 ng/ml with a lower detection limit of 0.1 ng/ml. The AFP level of healthy population showed a Gaussian distribution curve after logarithmic transformation with a median and 2.5-97.5 percentile reference range of 2.2 (0.6-5.6) ng/ml. There was no significant difference in the AFP level between males and females. Individuals with abnormal liver function tests alone showed no significant increase in serum AFP unless they were associated with positive hepatitis virus markers.

Key words: α-fetoprotein, enzyme immunoassay, healthy Japanese adults, serum level, reference values

α-fetoprotein (AFP) is an oncofetal glycoprotein which was discovered by Abelev *et al.* in 1963 (1) and its serum level increases in pregnant women as it is a normal serum protein during early fetal life. AFP concentration in serum decreases after being born, reaching undetectable levels in 300 days (2). Serum AFP level in adults rises in patients with hepatocellular carcinoma, yolk sac tumors and other malignancies (3). AFP also increases in serum of patients with hepatitis and cirrhosis of the liver (3), which are the premalignant conditions of hepatocellular carcinoma. The differentiation of elevated serum levels of AFP between hepatocellular carcinoma and benign liver diseases can be effected by analysis of the alteration of AFP sugar chains by means of lectin affinity electrophoresis (4). Alteration of AFP sugar chains in hepatocellular carcinoma with a small rise or even a normal level of AFP has been reported (5). Thus, the detection of significant changes in AFP at low levels in serum of patients with cirrhosis becomes important for early diagnosis of hepatocellular carcinoma. Therefore, accurate and precise determination of low AFP levels is needed to obtain reference ranges in defined populations of healthy adults.

Normal ranges of serum AFP have been reported by Ruoslahti and Seppälä (6) to be less than 10 ng/ml by means of a sensitive radioimmunoassay. Nishi and Hirai (3) also reported normal levels of AFP in Japanese to be below 10 ng/ml. However, the detection limit of those assays were not sufficiently low, leaving the lower reference value of AFP in healthy adults unsettled.

Sato *et al.* (7) determined the normal range to be $1.43 \pm 2 \times 0.83$ IU/ml (1 IU = 1.10 ng) by a sandwich radioimmunometric assay on an undefined group of healthy adults. Ball *et al.* (8) reported the normal level of serum AFP for well-defined adults to be 3.04 ± 1.9 ng/ml, which was determined by a sequential competitive radioimmunoassay in the liquid phase, although the lower normal range of the determination and the lower detection limit of the assay were close.

In the present study, we attempted to determine the reference range (previous normal range) of serum AFP in a Japanese population by an enzyme immunoassay with increased sensitivity. Known factors which would affect the AFP level, such as abnormal results of liver function tests or positive hepatitis virus markers, were also taken

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into account. This is probably the first comprehensive study on the serum AFP level of healthy Japanese adults.

Subjects and Methods

Subjects. Sera were collected from 616 healthy adults, 307 males and 309 females, ranging in age from 20 to 89 years old, at the time of periodical health checks performed by Health and Medical Center, Okayama University in 1996 and by the National Health Program for Aged People in Okayama prefecture from 1993 to 1996 after obtaining informed consent for analyzing AFP. They had normal results of liver function tests and were negative for hepatitis B surface antigen (HBsAg) and anti-hepatitis C virus antibodies (anti-HCV). Sera were also collected in the health check from 241 subjects with abnormal liver function tests, including aspartate aminotransferase (AST), alanine aminotransferase (ALT), γ-glutamyltransferase (GGT) and zinc turbidity test (ZTT), and/or positive virus markers of HBsAg or anti-HCV. Frozen sera were analyzed for AFP within three months.

Methods. Serum AFP concentrations were determined by means of an IMx Automated Immunoassay Analyzer using IMx AFP Dainapack kits from Dainabot Co., Tokyo Japan. The method was a sandwich enzyme immunoassay with monoclonal antibody bound to polymer microparticles. The bound AFP was separated from free particles with a glass fiber disc and allowed to react with alkaline phosphatase-labeled monoclonal antibody against AFP, followed by 4-methyl umbelliferyl phosphate as a substrate to measure the fluorescence of liberated 4-methyl umbelliferone at 448nm. The program for reaction system was set to give a two fold increase in sensitivity as compared with the routine laboratory assay of serum AFP by narrowing the calibration range. Human AFP Japanese Standard (9) (Nippon Bio-Test Laboratories, Tokyo, Japan) was included together with the AFP standard provided in the kit. AFP concentrations were also determined on some sera by Ohtsuka Assay Co., Tokushima, with an α-FETO-RIABEAD (Dainabot Co.,).

Statistical analysis. Results were given in means $\pm$ SD and in medians with 2.5–97.5 percentile ranges. Arithmetic and geometric means were calculated for comparison. Statistical significance between two groups was analyzed by the Student's t-test or Mann-Whitney test and that among multiple groups by the Kruskal-Wallis test using SPSS, Base System, SPSS Inc., Tokyo, Japan. The level of statistical significance was set at $P < 0.05$.

Results

In order to determine the lower detection limit of the present assay system as well as the accuracy and precision, ten determinations were made with the Japanese AFP standard, ranging in concentration from 0 to 50ng/ml. The results are given in Fig. 1, divided into two concentration ranges. There was good linearity in the overall concentration range of AFP tested. A statistically significant difference in AFP value was found between the 0 and 0.1ng/ml concentrations of AFP standard. However, the coefficient of variation given in the same figure became larger and exceeded 10 % at AFP concentrations below 0.1ng/ml. Accordingly, reproducible results were obtained at AFP concentrations equal to or greater than 0.2ng/ml. Linear regression analysis of simultaneous determinations showed $Y = 1.33 \times - 0.86$ ($n = 23$) for the Japanese AFP standard (Y) and $Y' = 0.84 \times - 0.08$ ($n = 11$) for determination with α-FETO-RIABEAD by Otsuka Assay Co. (Y'), where X stands for the value obtained by the IMx assay with the provided AFP standard.

Age-dependent distribution of serum AFP levels in individuals without abnormal results in health examinations is shown in Fig. 2. Males and females showed similar distributions with age. There was no tendency of AFP level to increase with age with a regression coefficient of less than 0.007ng/ml per year. The age-dependent medians and 2.5–97.5 percentile ranges given in Table 1 support the above observation, showing no statistically significant difference between males and females.

Accordingly, AFP values in all the age groups in Table 1 were combined and analyzed for frequency distribution. The results presented in Figs. 3 and 4 showed a Gaussian distribution curve after logarithmic transformation both in males and females. Although the distribution was slightly skewed toward higher values in males as compared with females, there was no significant difference between them. Geometric means and reference ranges calculated from mean $\pm$ 2SD after logarithmic transformation are given in Table 2 in addition to medians and 2.5–97.5 percentile ranges. Arithmetic means and SD values are also presented in Table 2 for comparison with previously reported results. Geometric means were lower

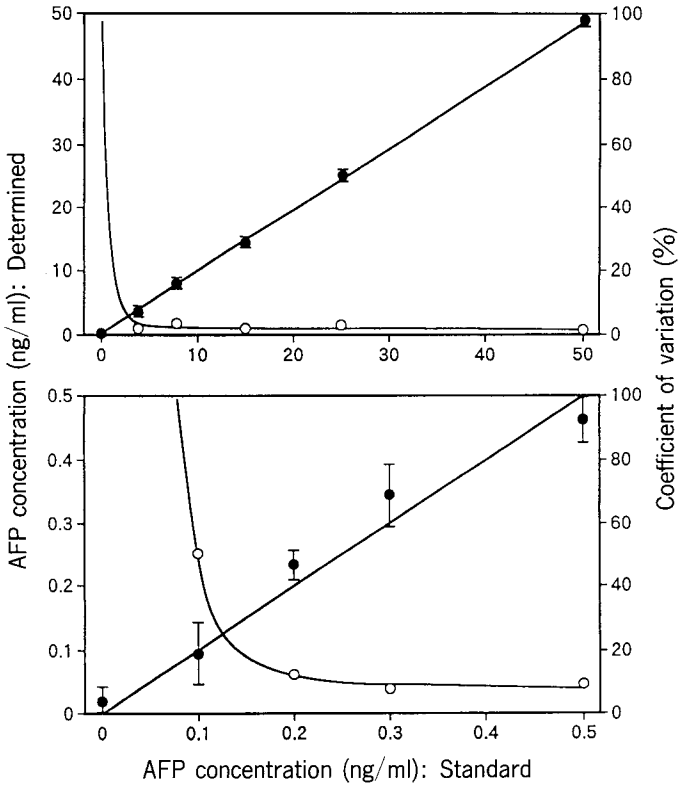

Fig. 1 α-fetoprotein (AFP) concentrations of standard solution in relation to determined values of AFP concentration together with coefficients of variation at different concentrations of AFP, showing the accuracy and precision of the present assay with the IMx assay system. ●: AFP concentration; ○: Coefficient of variation.

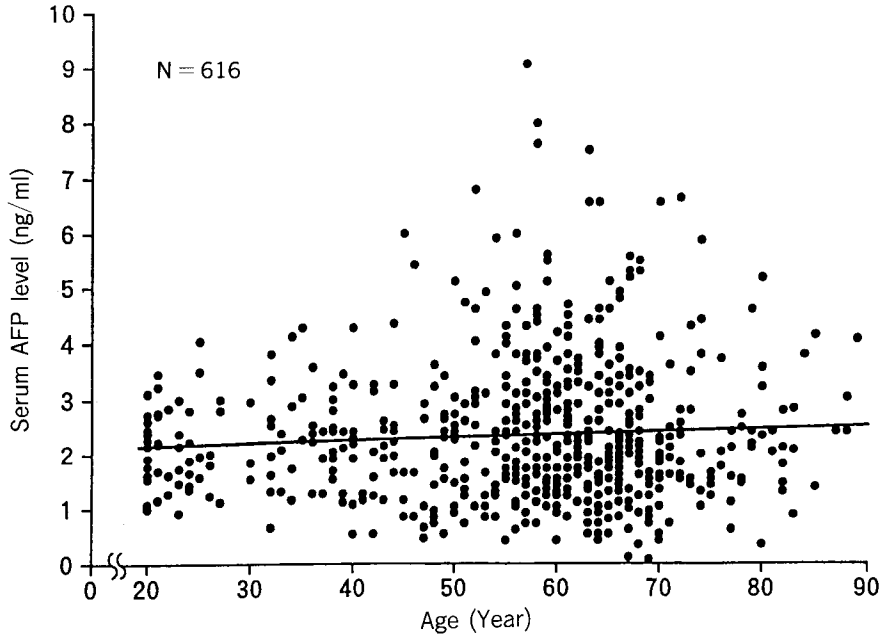

Fig. 2 Age-dependent distributions of serum α-fetoprotein (AFP) level in healthy subjects without abnormal results of liver function tests or virus markers. Males, 307; females, 309. The straight line is drawn for $Y = 0.0055X + 2.12$ (Y, AFP level; X, age).

Table I Age-dependent serum α-fetoprotein (AFP) levels

Sex	Age (Year)	Number of cases	AFP level (ng/ml)	
			Median	2.5-97.5 percentile
Males				
	20-29	26	2.4	1.1-3.6
	30-39	24	2.3	1.3-4.0
	40-49	34	2.0	0.6-3.8
	50-59	37	2.7	1.1-5.6
	60-69	130	2.2	0.5-5.1
	70-79	44	2.2	0.8-5.9
	80-89	12	2.7	0.7-4.2
Females				
	20-29	25	1.9	1.1-3.5
	30-39	22	2.3	1.0-4.0
	40-49	29	2.1	0.6-5.7
	50-59	110	2.4	0.8-7.1
	60-69	92	2.2	0.7-6.4
	70-79	20	1.7	0.5-5.4
	80-89	11	2.2	1.1 5.0
Total				
	20-29	51	2.2	1.0-3.5
	30-39	46	2.0	1.2-4.1
	40-49	63	2.1	0.6-4.9
	50 59	147	2.3	0.8-6.4
	60-69	222	2.2	0.6-5.4
	70-79	64	2.3	0.7-6.3
	80-89	23	2.5	0.7-4.7

and 2SD ranges were wider ranges than the medians and 2.5-97.5 percentile ranges. Arithmetic means were the largest.

Serum AFP values in healthy subjects were compared with those in groups of abnormal results of liver function tests and/or presence or absence of virus markers (Table 3). Anti-HCV-positive subjects had a significantly higher median AFP level irrespective of the results of liver function tests. Abnormal liver function tests alone were not associated with increased AFP level, although individuals who had abnormal liver function tests in combination with HBsAg positivity or anti-HCV showed significantly higher AFP levels. The median AFP level in anti-HCV-positive subjects was higher than that in HBsAg-positive ones.

Discussion

Serum AFP levels in healthy subjects have been reported by several researchers. The values differ slightly from one report to another, and whether the variation is due to the difference in the assay method or in the subjects from whom blood was collected, including ethnic difference, is unknown (9). Masseyeff *et al.* (10) reported a value of 2.6 ± 1.6 ng/ml by competitive radioimmunoassay with a lower detection limit of 0.1 ng/ml. Sato *et al.*

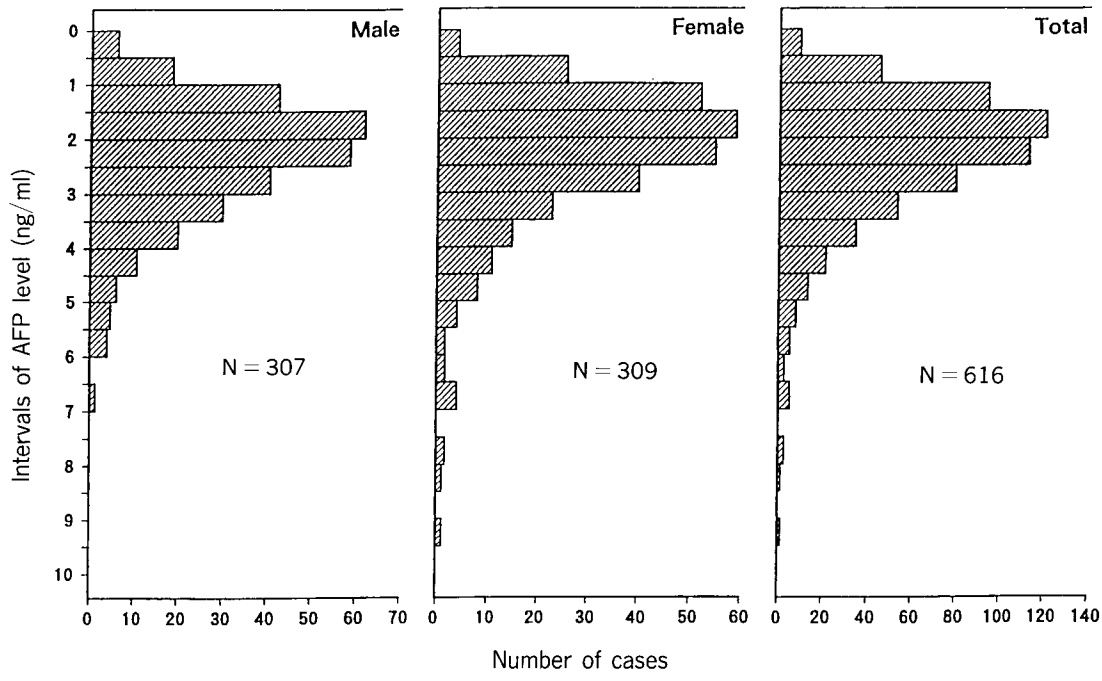

Fig. 3 Frequency distribution of serum α-fetoprotein (AFP) level in healthy males and females.

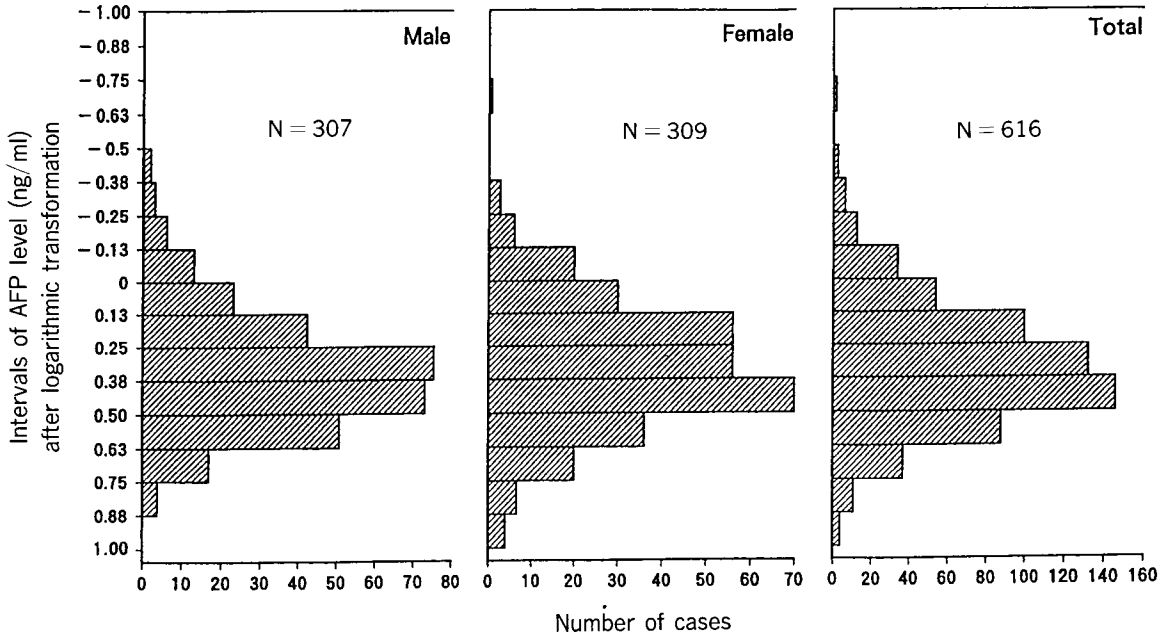

Fig. 4 Frequency distribution of serum α-fetoprotein (AFP) level in healthy males and females after logarithmic transformation of the AFP value.

Table 2 Statistical values of serum α-fetoprotein (AFP) levels

Sex	Means ± SD (Arithmetic)	Means (−2SD, +2SD) (Geometric)	Median (2.5–97.5 percentile)
Males	2.4 ± 1.1	2.1 (0.7–6.2)	2.2 (0.6–5.2)
Females	2.3 ± 1.2	2.1 (0.7–6.4)	2.2 (0.7–6.3)
Total	2.4 ± 1.1	2.1 (0.7–6.2)	2.2 (0.6–5.6)

Table 3 Serum α-fetoprotein (AFP) levels in subjects with or without abnormal results of liver function tests and/or virus markers

Liver function tests[a]	Number of cases	HBsAg	Anti-HCV	AFP Median (2.5–97.5 percentile)
Normal	616	−	−	2.2 (0.6–5.6)
Normal	19	+	−	2.5 (0.6–4.8)
Normal	43	−	+	2.5 (1.0–5.5)
Abnormal	56	−	−	2.2 (0.4–6.8)
Abnormal	41	+	−	3.2 (1.2–33.7)
Abnormal	82	−	+	4.3 (0.9–87.4)

*: $P < 0.05$; **: $P < 0.01$; ***: $P < 0.001$.

[a]: At least any one of the indicated liver function tests was abnormal (AST > 30IU/l, ALT > 30IU/l, GGT > 50mU/ml and ZTT > 12 KU).

HBsAg: Hepatitis B surface antigen; HCV: Hepatitis C virus; AST: Aspartate aminotransferase; ALT: Alanine aminotransferase; GGT: γ-glutamyltransferase; ZTT: Zinc turbidity test.

(7) obtained a much lower mean value of 1.31 ± 0.64 ng/ml by a sandwich radioimmunometric assay with a filter paper disc, having a sensitivity of 2.1 ng/ml. A more sensitive assay method of AFP has been developed recently by employing chemiluminescence in enzyme immunoassay (Luminomaster, Sankyo Co., Tokyo). Mean and SD values of 3.2 ± 1.18 (1.04–6.83) have been reported by this method which has a lower detection limit of 0.048 ng/ml (11).

Ball *et al.* (8) have reported the most reliable reference values on well defined subjects with a mean value of 3.04 ± 1.9 ng/ml with a significantly higher level in men than in women and a significant increase with age. The present study was similar to that of Ball *et al.* except that the sensitivity of our method with the IMx system was higher, with a lower detection limit of 0.1 ng/ml based on the difference from 0 ng/ml, than their method (which had a lower detection limit of 0.5 ng/ml). However, the coefficient of variation in our system at 0.1 ng/ml AFP was greater than 10 %. Accordingly, the lowest concentration of AFP to be detected with a sufficient accuracy by the IMx method was considered to be 0.2 ng/ml. On the other hand, the precision of our method leaves some questions to be answered in terms of the standardization of assay methods, because the AFP values determined with the Human AFP Japanese Standard (9) were 33 %

higher and those obtained with Dainabot RIA kit were 16 % lower than those determined by the IMx assay system with supplied standard solutions. If we take the Human AFP Japanese Standard as the gold standard, the reference values should be 33 % higher.

Taking these factors into account in interpreting the present reference range of 0.6–5.6 ng/ml with a median of 2.2 ng/ml in Japanese healthy adults, 10 ng/ml is reasonable to accept as a cutoff level for clinical purposes (12); however, it should never be above 10 ng/ml as it would miss those patients with slightly elevated AFP due to liver injuries, such as chronic hepatitis and liver cirrhosis, small well-differentiated hepatocellular carcinomas or early pregnancy. The lower cutoff level of 0.8 ng/ml would not be useful to delineate cases of congenital deficiency of AFP (13) without analyzing the pregnancy-induced level of AFP, because healthy subjects with AFP levels below 0.8 ng/ml would be present in 5 % of cases.

Another interesting result of the present study is the association of increased serum AFP level with hepatitis virus infection. Although abnormal liver function alone was not associated with elevated serum AFP levels, subjects with positive HBsAg or anti-HCV together with abnormal results of liver function tests showed significantly higher AFP levels. Interestingly, the AFP levels in anti-HCV-positive subjects were significantly high, irrespective of the results of liver function tests and subjects with abnormal liver function tests with positive anti-HCV had higher AFP level than those with positive HBsAg. This is compatible with the results of previous studies who showed that serum AFP in hepatitis B is characterized by spike-wise elevations with mostly normal base line levels, while serum AFP in hepatitis C shows wave-like fluctuations at slightly increased levels above the cutoff value (14, 15). Further studies of serum AFP levels in chronic hepatitis and cirrhosis patients are obviously needed. Since cirrhotic patients with elevated serum AFP are at a higher risk of developing hepatocellular carcinoma than those without (16) and HCV-positive cirrhotics tends to develop into hepatocellular carcinoma at a higher rate (17), increased AFP levels in the virus marker-positive subjects in a community-based population is a warning of future evolution into hepatocellular carcinoma and proper intervention should be considered from the view point of public health.

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