Alpha-feto protein radioimmunoassay and its values for the diagnosis of hepatoma among normal Japanese population and those with chronic schistosomiasis

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Since its first report made by Ablev et al., α-feto protein which appears both in fetal serum and serum in the cases with hepatoma drew attention of hepatologist in order to establish an qualitative method to diagnose the presence of hepatoma. Since conventional liver scintigraphy is not able to make the diagnosis of the nature of lesion which appears as space occupying lesion need for the more qualitative method facilitated the establishment of the method to detect the presence of α-feto protein in serum.

α-feto protein in the serum of hepatoma cases was reported to be identical to fetal α-feto protein by the immunological method.

At first, assay of the α-feto protein was performed using the precipitation reaction in agar gel and became quite popular among clinical hepatologists. Detection sensitivity of this conventional method, however, is approximately 10 μgr/ml and α-feto protein was detected in 80% of hepatoma patient. However detection percentage of the hepatoma cases associated with chronic schistosomiasis was found to be as poor as 30% which was measured by our group at Kofu municipal Hospital. Because of the frequent incidences of deformed & small liver by scintigraphy among cases with chronic schistosomiasis and these abnormal liver scintigraphy easily lead one to misdiagnose the presence of hepatoma[1], establishment of more sensitive assay method is needed.

After the success of purification and crystallization of the α-feto protein by the group of Univ. of Hokkaido[2], the radioimmunoassay technique was developed in Japan[2] and α-feto-125 Kit is now became available commercially*. Initial trial of this new radioisotope method was proven to be quite promising and this double antibody technique enabled us to measure 2 mμgr/ml of α-feto protein which was almost 500~1,000 times more sensitive than those of the conventional method.

Material and Method

Twenty six cases with hepatoma, 8 cases with acute hepatitis, 8 cases with chronic hepatitis, 10 cases with liver cirrhosis, 4 cases with cholelithiasis and 2 other cases and 50 normal control from Univ. of Tokyo Hospital, Tokyo Metropolitan Geriatrics Hospital and Kofu Municipal Hospital were examined. Among 26 cases with hepatoma, 13 cases were from Tokyo district and the rest of 13 cases were from Kofu district. The latter cases were associated with chronic schistosomiasis japonicum. Histological examina-

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tion was performed on these cases to illucidate the presence of cirrhosis as basic disorder. Liver scintigraphy was performed on all cases examined. Final diagnosis of hepatoma was made by autopsy, or peritoneoscopy, or liver biopsy and necropsy.

Result

Since almost all normal cases has serum α-feto

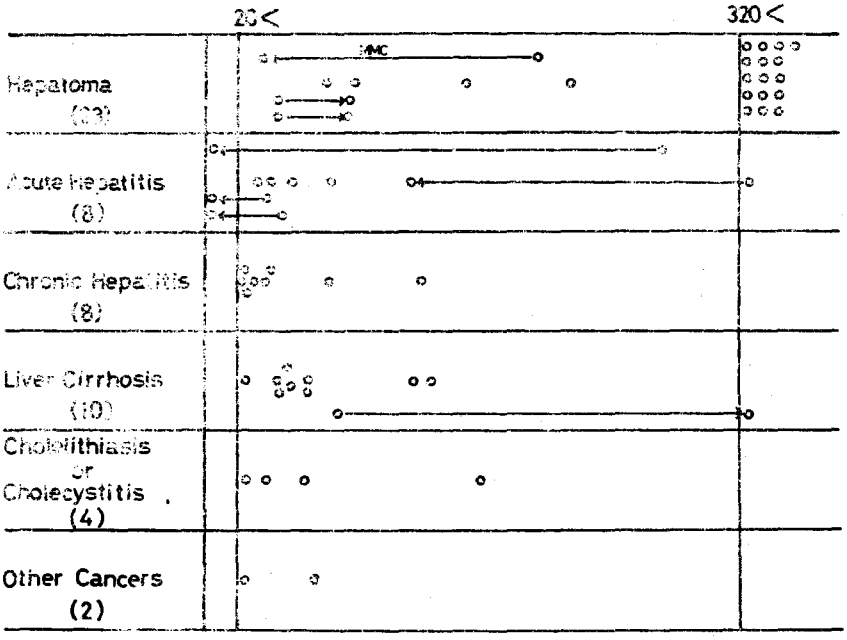

Fig. 1. Cases with serum α-feto protein over 20 mμg/ml.

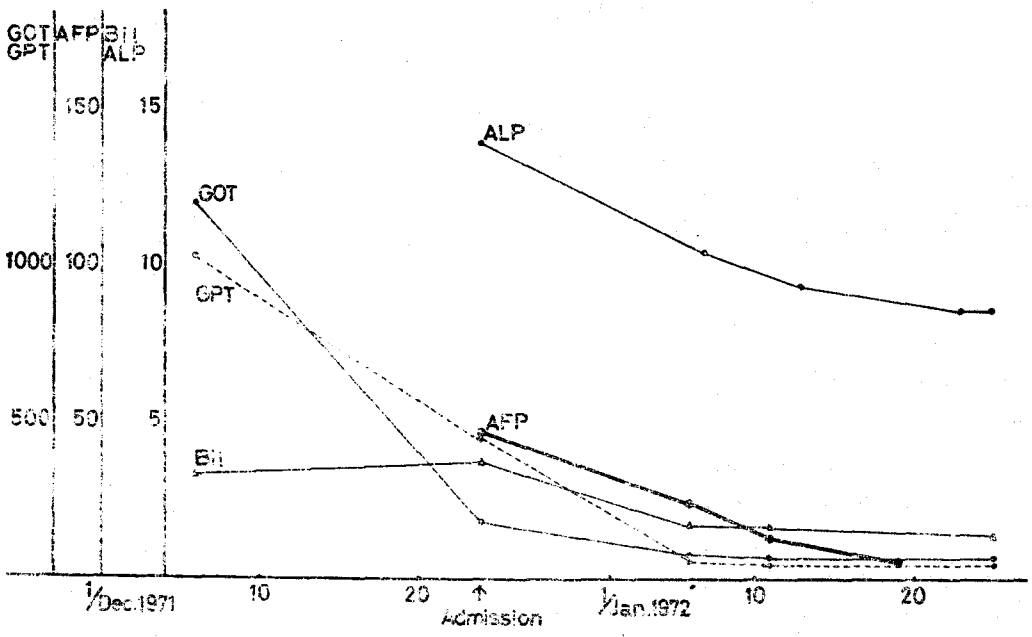

Fig. 2. Serum α-feto protein in hepatitis.

protein less than 10 mμgr/ml with some exception who has α-feto protein value ranging more than 10 mμgr and less than 20 mμgr/ml, 20 mμgr/ml was defined as normal upper range. As is shown in Fig. 1 increased values of α-feto protein were found in various liver disorders ranging mostly between 20～320 mμgr/ml except cases with hepatoma. A case with hepatitis who showed marked increase (more than 320 mμgr/ml) is a case with severe hepatitis associated with pregnancy. Along with the improvent of hepatitis her α-feto protein level decreased below 320 mμgr/dl. During the continuous observation cases with hepatoma show increase in α-feto protein in the serum, whereas cases with hepatitis show continuous decrease. A case with liver cirrhosis who showed marked increase in α-feto protein is a case admitted to hospital by a sudden incidence of hepatic insufficiency during consultation to the outpatient clinic. Complication of hepatoma in this case is not proven at this stage.

Time course of α-feto protein level in hapatitis is shown in the Fig. 2. Continuous decrease of the serum level of α-feto protein is observed as the other liver function tests became improved. This phenomenon is used to differenciate hepatoma from other reversible liver diseases by using sometimes too sensitive α-feto protein radioimmunoassay techique.

Twenty cases with primary hepatoma were found to have marked increase of α-feto protein with more than 320 mμgr/ml in 16 cases (61.5 %), moderate increase of 20～30 mμgr/ml in 7 cases (26.9%) and negative in 3 cases (11.6%). Negative 3 cases have normal value of below 10 mμgr/ml on repeated examinations and these cases can be defined as α-feto protein non-producing hepatomas (Fig. 3). One case who are treated by mytomycin showed decrease in α-feto protein.

When further analysis was made by separating group of patients according to basic disorders following results could be drawn (Table 1). Among 13 hepatoma cases from Tokyo district 12 cases (92%) showed α-feto protein serum level more than 20 mμgr/ml. Whereas among 13 hepatoma cases from Kofu district 11 cases (84%) also showed α-feto protein level above normal. This is an improvement of the detection rate of hepatoma from 80% to 92% in Tokyo and more markedly from 30% to 84% in Kofu by changing the technique from agar gel diffusion method to recently introduced radioimmunoassay. Among these positive cases 10 cases (77%) from Tokyo district

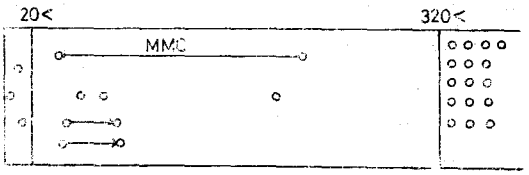

Fig. 3. Serum α-feto protein in hepatoma.

Table 1. Serum α-feto protein level in hepatoma

	Hepatoma in Tokyo	Hepatoma in Kofu	Total
Over 320 mμg	10(77%)	6(46%)	16(61.5%)
20～320 mμg	2(15%)	5(38%)	7(26.9%)
Below 20 mμg	1 (8%)	2(16%)	3(11.6%)
Total cases	13	13	26
Detectability by Ria.	92%	84%	88.4%

Ria.: Radioimmunoassay

Table 2. α-feto protein VS liver scan

	Hepatoma in Tokyo	Hepatoma in Kofu
Positive scan	8	12
Negative scan	1	1
Total	9	13

Negative case in Tokyo: α-feto protein 320,000<
Negative case in Kofu: α-feto protein 200 mμg/ml scan showed SOL 2 month later.

showed marked increase of more than 320 mμgr/ml, whereas only 6 cases (46%) from Kofu district showed such significant increase in α-feto protein serum level. These facts explain why we had poor result by conventional immunodiffusion method.

These facts also suggest the necessity to perform repeated liver scintigraphy, peritoneoscopy and angiography on such cases who showed slight increase in α-feto protein serum level.

As is shown in Table 2 90% positive scan with SOL were found among cases with positive α-feto protein serum level. A case with negative scan from Tokyo district is a case with marked increase in α-feto protein serum level and associated with liver cirrhosis. A case with negative scan from Kofu district is a 57 y.o. female who showed almost normal scan on Sept. 20th. However moderately increased α-feto protein serum level of

Table 3. Serum α-FP association of liver cirrhosis

Serum α-FP level	Hepatoma in Tokyo		Hepatoma in Kofu	
	with l.c.	without l.c.	with l.c.	without l.c.
320 mμg<	9	1	5	1
20~320 mμg	1	1	0	5
<20 mμg	1	0	0	2

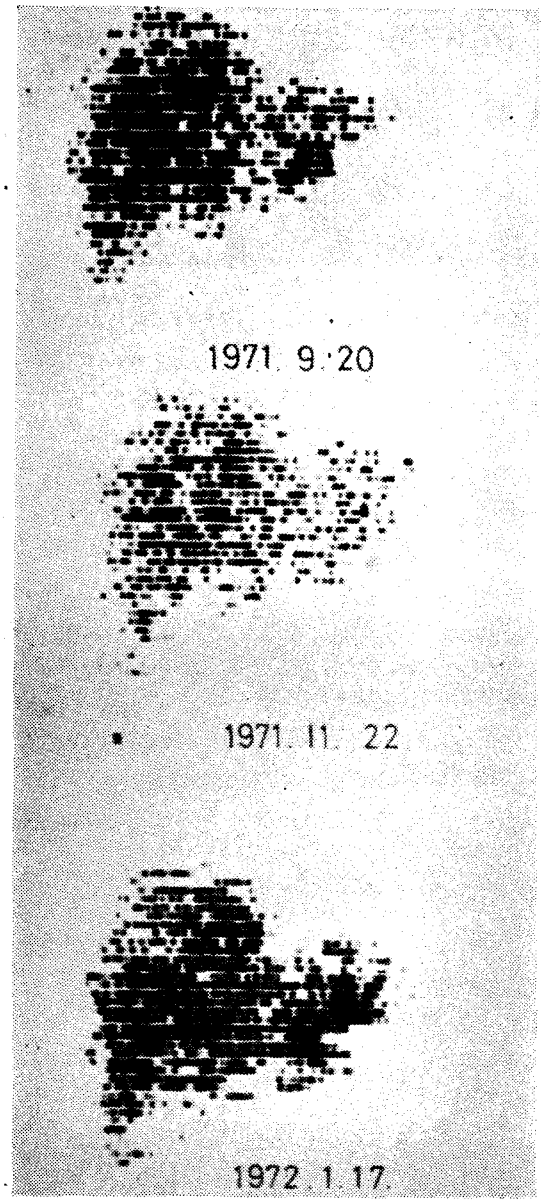

Fig. 4.

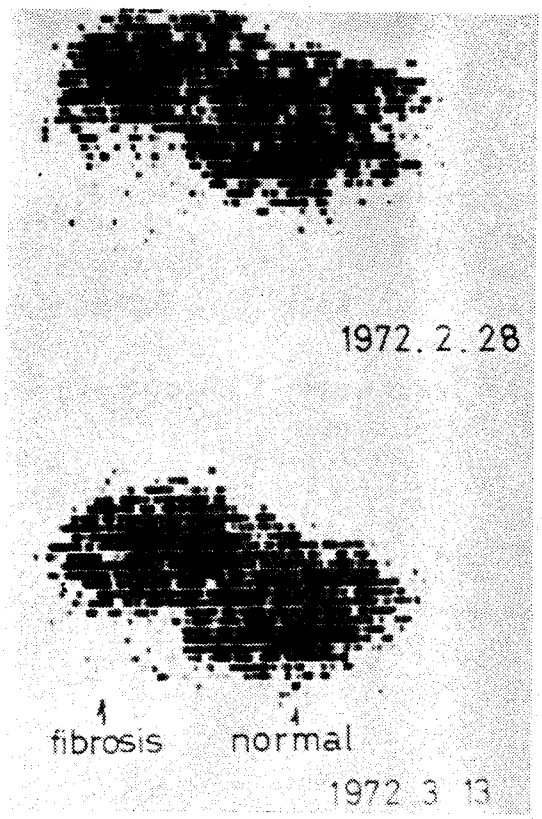

Fig. 5.

220 mμgr/ml made us to follow up the case by repeated scans. Three months later clear-cut space occupying lesion at the left upper border of the liver became to be seen clearly and was proven to be hepatoma in nature(Fig. 4).

After the initial observation of difference in the production of α-feto protein in hepatoma cases with and without schistosomiasis, authors evaluated the group further histologically. Table 3 indicates the result. It has become apparent that cases with hepatoma in Tokyo district accompany mostly with liver cirrhosis and have increased serum α-feto protein level. Whereas cases from Kofu with schistosomiasis have increased α-feto protein level if liver cirrhosis is present and have slight increase in α-feto protein if liver cirrhosis is not present as basic disorders.

Fig. 5 is a 57 year old house wife infected by schistosoma. Liver scintigram showed a highly suspicious huge space occupying lesion which occupied lower portion of right lobe. α-feto protein of this case revealed to remain within normal range. Follow up scan after 3 months of initial scan showed no progress of the lesion and the patient remained without complaint. At biopsy fibrotic change of right lobe was found.

Discussion

Significantly improved sensitivity of the method of α-feto protein serum level by the introduction of radioimmunoassay enabled us to make more sophisticated use of the serum α-feto protein level in various clinical conditions including hepatoma, hepatitis, pregnancy and other disorders. Not only hepatoma but the other conditions will produce a slight amount of α-feto protein in the serum, however, time course usually revealed these non-hepatoma cases to be different from hepatoma cases who showed continuous increase in the level of serum α-feto protein. It is interesting that even normal control produces slight amount of α-feto protein (less than 10 mμgr/ml) which can be removed by immunoadsorbent column consisted from anti-alfa-feto protein horse IgG coupled to Sepharose 6B by BrCN Method of Porath.

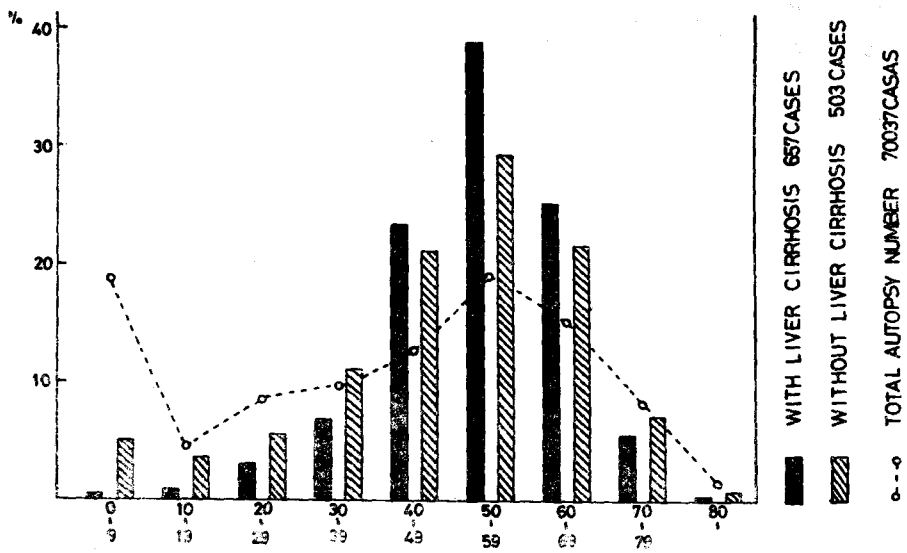

Fig. 6. Association of liver cirrhosis in liver cancer in Japan(1958~1963) (From "The liver" ed. by T. Takahashi)

In our observation, it is concluded that low percentage of positive α-feto protein test among cases with schistosomiasis by conventional method was due to the lack of sensitivity the of method to the cases who produced usually only a slight amount of α-feto protein when compared with hepatoma cases from other district. Also it was found that these cases fell in the group who has no complication of liver cirrhosis as its basic disorders.

Statistic study on the incidence of hepatoma in Japan revealed (by Takahashi) that peak of the incidence was on the 50th decade and then it decreased rapidly according to further aging(Fig. 6). There is no marked difference in the incidence between hepatoma cases with liver cirrhosis and those with out liver cirrhosis.

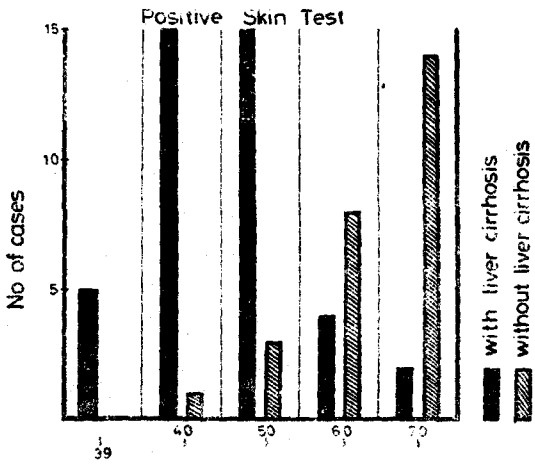

Fig. 7. Age difference in association of liver cirrhosis with hepatoma in 66 hepatoma case with positive skin test.

When compared with this, incidence of hepatoma at Kofu district, where schistosomiasis dominates, showed no definite tendency of decrease in the incidence of hepatoma according to the aging. Hepatoma without liver cirrhosis, on the contrary, showed rather definite increase from 40th to 70th decade. Low level of α-feto protein producing type hepatoma usually fell in this group (Fig. 7).

Newly introduced α-feto protein radioimmunoassay expands the knowledge of clinical hepatology much more than that we had several years ago. However, because this method still requires 2 days incubation and tedious manupulations, we are now in the process of simplifying the method by introducing single well isotope diffusion method. It is hoped that we could present the result of this progress in near future to the friendly audience of our neighbour country.

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