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Serum alpha-fetoprotein levels in patients with acute and chronic liver disease

Relation to hepatocellular regeneration and development of primary liver cell carcinoma

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SUMMARY Using a radioimmunoassay technique serum alpha-fetoprotein could be detected in healthy adults in concentrations of less than 20 μg/l.

Of patients with acute, viral hepatitis 43% exhibited a transient rise of serum alpha-fetoprotein, the peak occurring eight to nine days after the maximum recorded serum aspartate transaminase activity. Patients with hepatic damage due to paracetamol poisoning were also shown to have transiently raised levels, the peak occurring earlier than in subjects with viral hepatitis. Six subjects with fatal fulminant hepatitis were studied; the three with the more protracted illness were noted to have increased levels before death.

Twenty of 163 cases of chronic liver disease also had raised serum alpha-fetoprotein concentrations. In four, primary liver cell cancer developed; in two of these the serum alpha-fetoprotein levels rose progressively, and in two it remained raised but at low levels.

Alpha-fetoprotein is an alpha globulin produced by the fetal liver which disappears from the serum within three weeks of birth. Tatarinov (1964) described the association of primary liver cell carcinoma with the appearance of alpha-fetoprotein in the serum, and this finding has been confirmed by others (Abelev, 1968; Alpert *et al.*, 1968; Smith and Todd, 1968; Foli *et al.*, 1969; Kew, 1974). The original method of detection was the relatively insensitive technique, gel diffusion. More recently, radioimmunoassay techniques have shown that alpha-fetoprotein can be detected in healthy adults in concentrations of less than 20 μg/l (Purves and Purves, 1972; Adinolfi *et al.*, 1975). Using this highly sensitive method, patients with acute and chronic liver disease have also shown increased values (Smith, 1971; Akeyama *et al.*, 1972; Kew *et al.*, 1973; Silver *et al.*, 1974; Bloomer *et al.*, 1975). It has been suggested that liver cell regeneration may be responsible for the rises in serum alpha-fetoprotein in these patients with non-malignant liver disease (Silver *et al.*, 1974; Bloomer *et al.*, 1975).

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We have carried out serial serum alpha-fetoprotein determinations in subjects with acute hepatic damage in order to assess the value of this test as a marker of hepatocellular regeneration. Subjects with chronic liver disease were also examined, in particular to determine whether this highly sensitive method of alpha-fetoprotein determination was of value in predicting the development of primary liver cell carcinoma.

Patients studied

Control sera came from 50 healthy members of the hospital staff.

The patients with acute liver disease suffered from acute, non-fulminant viral hepatitis (44), paracetamol overdose (eight, of whom three received cysteamine), and fulminant hepatitis (six, five presumed viral and one halothane-related); serial blood samples were taken, usually every two to four days.

One hundred and sixty-three patients with biopsy-proven chronic liver disease were studied. They comprised primary biliary cirrhosis (47), cryptogenic cirrhosis (16), alcohol-related liver disease (44), and chronic active liver disease either of the 'lupoid' variety or associated with serum hepatitis B surface antigen (HB_SAg) (56).

Methods

All sera were tested for the presence of alpha-fetoprotein using the standard counter-immuno-electrophoresis (CIEP) technique employing commercial alpha-fetoprotein antibody. Serum concentrations of <500 μg/l were not detected by this method.

The Abbott radioimmunoassay kit employs the double antibody technique and was used on all the sera, the procedure being carried out according to the manufacturer's instructions. This method detected serum alpha-fetoprotein levels of 5 to 320 μg/l. When levels of alpha-fetoprotein greater than 320 μg/l were detected, the test was repeated after the sera had been diluted with normal saline to 1:100 or more.

All sera were also tested for the presence of hepatitis B surface antigen (HB_SAg) using the direct haemagglutination test (Burroughs Welcome—Hepatest).

Serum aspartate transaminase activity was determined on all sera.

Results

HEALTHY ADULTS

The serum specimens obtained from 50 healthy adults all had alpha-fetoprotein concentrations of <20 μg/l and were negative by CIEP.

ACUTE VIRAL HEPATITIS

Fifteen of the 44 patients studied were HB_SAg positive.

Nineteen of the 44 patients showed a rise in serum alpha-fetoprotein while in hospital, nine of whom were HB_SAg positive. The rise was recorded eight to nine days after the maximum aspartate transaminase activity. The mean peak level was 80 μg/l in those who were HB_SAg positive and 92 μg/l in those who were HB_SAg negative. The serum alpha-fetoprotein had returned to normal values in 15 patients when they left hospital, and in the other four, levels were falling although they were still raised.

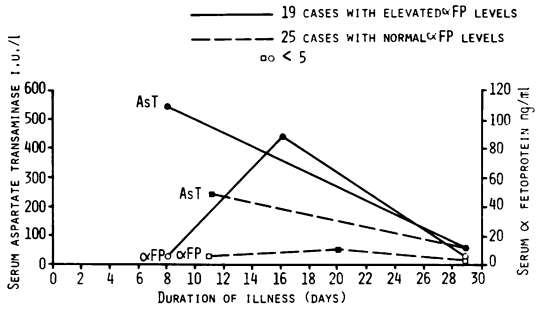

Fig. 1 *Acute viral hepatitis: relationship of mean maximum serum aspartate transaminase and serum alpha-fetoprotein level to duration of illness.*

The mean maximum aspartate transaminase in those with raised alpha-fetoprotein levels was 549 IU/litre (range 840 to 300), whereas the mean maximum recorded aspartate transaminase in the others was 285 IU/litre (range 390 to 105) (normal range <17 IU/l).

The mean duration of symptoms before admission to hospital was comparable in the two groups, namely, 9·4 days in those with raised alpha-fetoprotein levels and 11·7 days in those whose serum alpha-fetoprotein remained within the normal range. Similarly, the average hospital stay was comparable—20 and 17 days respectively (Fig. 1).

PARACETAMOL POISONING

Five of the eight patients, two of whom had been give cysteamine, developed a raised serum alpha-fetoprotein. In these five the peak serum alpha-fetoprotein level ranged from 40 to 450 μg/l and was seen 2-10 days after paracetamol ingestion. In three of the five the highest serum alpha-fetoprotein values were noted at 7-10 days, being their last day in hospital. In the other two (both of whom had been given cysteamine) the serum alpha-fetoprotein peak occurred two days after ingestion and the levels were seen to fall to normal by the time of discharge. All but one of these subjects showed a rise in serum aspartate transaminase activity (Table 1).

Table 1 *Paracetamol poisoning*

Cysteamine	*Case*	**Maximum serum AST (IU/l)*	*Peak serum αFP (μg/l)*
Yes	1	10	42
,,	2	23	<5
,,	3	20	40
No	4	1550	190
,,	5	179	87
,,	6	100	450
,,	7	86	<5
,,	8	40	<5

*Normal values <17 IU/l

FULMINANT HEPATITIS

All six cases of fulminant viral hepatitis died within seven days of admission. In three, serum alpha-fetoprotein levels remained within normal limits until death at two, three and seven days. Serum alpha-fetoprotein values were above normal in the three other cases, the peak range being 50-112 μg/l (Table 2). In two, the levels fell but did not reach normal values before death. The average duration

Table 2 *Fatal fulminant hepatitis*

Case	*Duration of symptoms (days)*	**Peak serum AST (IU/l)*	*Peak serum AFP (μg/l)*
1	13	820	4
2	5	1020	4
3	17	500	4
4	4	76	112
5	56	362	50
6	21	32	112

*Normal values <17 IU/l

of illness was 27 days for those with raised serum alpha-fetoprotein and 12 days in those with normal values. HB_SAg was detected in only one of the six patients.

CHRONIC LIVER DISEASE (Fig. 2)

Primary biliary cirrhosis

Sixty-five serum samples were obtained from the 47 patients, 14 subjects being examined more than once. Sixty-two of the sera gave normal alpha-fetoprotein values. One patient tested only once had a serum alpha-fetoprotein level of 28 μg/l, and another, found initially to have a serum alpha-fetoprotein level of 17 μg/l some four months later, had a level of 55 μg/l and, a further six months later, 65 μg/l. Both these patients remain clinically unchanged six months later. All 47 patients were HB_SAg negative.

Cryptogenic cirrhosis

Twenty-two serum samples were tested from 16 patients, and 17 samples gave normal alpha-fetoprotein concentrations. One patient with normal serum alpha-fetoprotein values on two separate occasions, 18 months later had a serum alpha-fetoprotein value of 45 μg/l which fell 10 days later to 27 μg/l. Another patient had a serum alpha-fetoprotein level of 38 μg/l. A third subject had a serum alpha-fetoprotein value of 25 μg/l, which some 10 months later had risen to 45 μg/l. All 16 patients were HB_SAg negative.

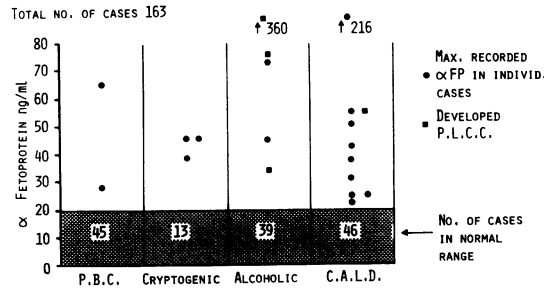

Fig. 2 *Serum alpha-fetoprotein levels in 163 patients with chronic liver disease: PLCC = primary liver cell carcinoma; PBC = primary biliary cirrhosis; CALD = chronic active liver disease.*

Alcoholic liver disease

Sixty-two sera from 44 patients were tested. In 12 sera from five patients the values exceeded 20 μg/l. One patient, found initially to have a serum alpha-fetoprotein level of 37 μg/l, two months later had a value of 73 μg/l and six months later 60 μg/l; he has remained clinically unchanged for the six months since the last estimation. Another patient had an initial value of 34 μg/l, falling four months later to 22 μg/l. Six months later a primary liver cell carcinoma was found at necropsy. One further patient had a serum alpha-fetoprotein value of 75 μg/l and eight months later was found to have a primary liver cell carcinoma at necropsy. A fourth subject, with a serum alpha-fetoprotein level of 360 μg/l, five months later showed a rise to 5000 μg/l. Three months later he died, his serum alpha-fetoprotein concentration being 50 000 μg/l at this time; a necropsy showed a primary liver cell carcinoma.

One subject tested on three separate occasions had a transient rise of serum alpha-fetoprotein to a level of 45 μg/l, but seven months later the level was within normal limits.

One of the 44 patients was HB_SAg positive.

Chronic active liver disease

Eighty-two sera from 56 subjects were tested. Raised levels were found in 10 subjects. Two subjects with levels of 22 and 42 μg/l have not been retested. In three patients the rise was transient, the maximum levels being 25 μg/l, 55 μg/l, and 25 μg/l. Three other patients whose initial serum alpha-fetoprotein levels were normal, six weeks to eight months later showed levels ranging from 31 to 50 μg/l. No subsequent serum specimens have been obtained.

One patient who was HB_SAg positive had an initial value of 55 μg/l, which progressively rose over eight months to 650 000 μg/l; a highly vascular space-occupying lesion was found in the liver and *post mortem* histological confirmation of primary liver cell carcinoma was obtained. Another patient, whose initial serum alpha-fetoprotein was 216 μg/l, has shown progressively falling values to 60 μg/l. This patient died of liver failure with no evidence at necropsy of carcinoma.

Seventeen of these 56 patients were found to be HB_SAg positive, three of whom were found to have raised serum alpha-fetoprotein levels.

One hundred and ninety-one transaminase estimations were done on the 163 patients with chronic liver disease, believed to be without superimposed primary liver cell carcinoma. No correlation could be

found between aspartate transaminase level and serum alpha-fetoprotein (r = 0·09). In none of these sera could alpha-fetoprotein be detected using the CIEP test. Alpha-fetoprotein could be detected by both CIEP and radioimmunoassay in six later sera obtained from two of the four subjects who developed a primary liver cell carcinoma during the survey.

Discussion

Using radioimmunoassay, we have confirmed that alpha-fetoprotein can be detected in the serum of normal, healthy adults in concentrations of less than 20 μg/l. Serum concentrations greater than this but less than 500 μg/l were detected in patients with non-malignant acute and chronic liver disease. The lower limit of sensitivity of the standard CIEP test for detecting serum alpha-fetoprotein seems to be 500 μg/l, and this test became positive only in patients who developed primary liver cell carcinoma.

Patients with acute, non-fulminant viral hepatitis, both type B and non B studied serially, showed a rise above normal of serum alpha-fetoprotein. This transient rise occurred once the serum transaminase levels started to fall, the serum alpha-fetoprotein levels returning to normal by the time of discharge from hospital. Serum studies made before and after partial hepatectomy experiments in animals have indicated that hepatocyte proliferation is related to alpha-fetoprotein production (Sell *et al.*, 1974). It seems likely that the increased serum alpha-fetoprotein concentrations found a week or more after the onset of an acute hepatitis also reflect hepatic regeneration. However, in about half the subjects studied, the serum alpha-fetoprotein did not rise. The mean duration of illness before admission was about the same as in those with an increased serum alpha-fetoprotein. It is therefore unlikely that any transient rise was missed. The peak serum transaminase activity in those with normal serum alpha-fetoprotein levels tended to be lower than in those exhibiting an increase; it is possible that the smaller degree of liver cell necrosis was followed by less liver cell regeneration, which may explain the absence of an increase in alpha-fetoprotein concentration.

The observations in the paracetamol group suggest a correlation between degree of liver cell necrosis as judged by serum transaminase measurements and subsequent alpha-fetoprotein production. In these patients a rise in serum alpha-fetoprotein was recorded within 48 hours of ingestion of the drug, much sooner than in viral hepatitis. This may reflect the faster evolution of hepatic damage after paracetamol ingestion than after viral hepatitis. Patients given cysteamine within a few hours of paracetamol ingestion are protected from some of the hepatotoxic activity of the drug (Prescott *et al.*, 1974), and in general this was reflected in our study by lower aspartate transaminase and also alpha-fetoprotein levels. Nevertheless two patients given cysteamine did have a small increase in alpha-fetoprotein in spite of normal or only slightly increased transaminase levels. In these cases it is probable that some hepatic necrosis occurred but was not reflected in the standard liver function tests. These data are also consistent with the hypothesis that the rise in alpha-fetoprotein is due to increased synthesis of the protein by regenerating hepatocytes.

Half the patients with fatal fulminant hepatitis developed raised serum alpha-fetoprotein levels. The duration of symptoms before admission was much greater in those patients than in those whose serum alpha-fetoprotein levels remained within normal limits. A raised serum alpha-fetoprotein during the course of fulminant hepatitis has been said to be a favourable prognostic sign (Karvountzis and Redeker, 1974). Our findings are more in agreement with those of Bloomer *et al.* (1975) and suggest that the increase in serum alpha-fetoprotein in fulminant hepatitis is related to the duration of illness regardless of the final outcome.

Raised levels of serum alpha-fetoprotein greater than 20 μg/l but generally less than 500 μg/l were seen in a wide variety of chronic liver disease. These rises were often transient. No correlation existed between serum transaminase activity and alpha-fetoprotein measured at the same time. It seems probable that these fluctuating changes are a reflection of synthesis by regenerating hepatocytes, although direct proof of this is still lacking. Steadily increasing levels are highly suspicious of the development of primary liver cell carcinoma.

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References

Abelev, G. I. (1968). Production of embryonal serum α-globulin by hepatomas: review of experimental and clinical data. *Cancer Research*, **28**, 1344-1350.

Adinolfi, A., Adinolfi, M., and Lessof, M. H. (1975). Alpha-feto-protein during development and in disease. *Journal of Medical Genetics*, **12**, 138-151.

Akeyama, T., Koyama, T., and Kamada, T. (1972). Alpha-feto-protein in acute viral hepatitis (Letter). *New England Journal of Medicine*, **287**, 989.

Alpert, M. E., Uriel, J., and De Nechaud, B. (1968).

Alpha$_1$ fetoglobulin in the diagnosis of human hepatoma. *New England Journal of Medicine*, **278**, 984-986.

Bloomer, J. R., Waldmann, T. A., McIntire, K. R., and Klatskin, G. (1975). Relationship of serum α-fetoprotein to the severity and duration of illness in patients with viral hepatitis. *Gastroenterology*, **68**, 342-350.

Foli, A. K., Sherlock, S., and Adinolfi, M. (1969). Serum alpha-1-fetoprotein in patients with liver disease. *Lancet*, **2**, 1267-1269.

Karvountzis, G. G. and Redeker, A. G. (1974). Relation of alpha-fetoprotein in acute hepatitis to severity and prognosis. *Annals of Internal Medicine*, **80**, 156-160.

Kew, M. C. (1974). Alpha-fetoprotein in primary liver cancer and other diseases. *Gut*, **15**, 814-821.

Kew, M. C., Purves, L. R., and Bersohn, I. (1973). Serum alpha-fetoprotein levels in acute viral hepatitis. *Gut*, **14**, 939-942.

Prescott, L. F., Newton, R. W., Swainson, C. P., Wright, N., Forrest, A. R. W., and Matthew, H. (1974). Successful treatment of severe paracetamol overdosage with cysteamine. *Lancet*, **1**, 588-592.

Purves, L. R. and Purves, M. (1972). Serum alpha-fetoprotein. VI. The radio-immunoassay evidence for the presence of AFP in the serum of normal people and during pregnancy. *South African Medical Journal*, **46**, 1290-1297.

Sell, S., Nichols, M., Becker, F. F., and Leffert, H. L. (1974). Hepatocyte proliferation and α-fetoprotein in pregnant, neonatal, and partially hepatectomized rats. *Cancer Research*, **34**, 865-871.

Silver, H. K. B., Gold, P., Shuster, J., Javitt, N. B., Freedman, S. O., and Finlayson, N. D. C. (1974). Alpha$_1$-fetoprotein in chronic liver disease. *New England Journal of Medicine*, **291**, 506-508.

Smith, J. B. (1971). Occurrence of alpha-fetoprotein in acute viral hepatitis. *International Journal of Cancer*, **8**, 421-424.

Smith, J. B. and Todd, D. (1968). Foetoglobin and primary liver cancer (Letter). *Lancet*, **2**, 833.

Tatarinov, Y. S. (1964). Detection of embryospecific alpha-globulin in the blood serum of a patient with primary liver cancer. *Voprosy Meditsinskoi khimil*, **10**, 90-91.