THE ALPHA-FOETOPROTEIN TEST FOR LIVER CANCER

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An embryo-specific protein, alpha$_f$-globulin, was demonstrated by Abelev *et al* (1963) in the serum of mice bearing chemically induced hepatoma, and by Tatarinov *et al* (1964) in human patients with liver cancer. Recent reports have indicated that this globulin, identified as an alpha$_1$-foeto-specific protein (AFP), is present in the serum of some 50-80% of patients with hepatocellular carcinoma (Abelev *et al*, 1967; Tatarinov, 1965; Uriel *et al*, 1968; Purves *et al*, 1968). The alpha-foetoprotein test has also been shown to have a high degree of specificity for liver cell carcinoma. It is consistently negative in normal adults, in patients with various non-neoplastic liver diseases, and in patients with other cancers including those that have metastasised to the liver. The only other diseases in which the presence of alpha-foetoprotein has been reported are embryonal carcinomas, neonatal jaundice and hepatopathies of infancy (Uriel *et al*, 1968; Masopust *et al*, 1968).

This protein, normally present in the serum of embryos, was first discovered in the calf (Pedersen, 1944) and has subsequently been demonstrated in the foetuses of man (Bergstrand and Czar, 1956) and other animals. It is produced by foetal liver cells and is not found in the sera of normal adults. Several animal species have more than one foetoprotein. The protein that is associated with human hepatocellular carcinoma is an alpha$_1$-foetospecific serum protein (alpha-foetoprotein, AFP, post-albumin, embryospecific alpha-globulin); this protein is analogous to the alpha$_1$-foetospecific serum protein in monkeys and rats (LA, alpha$_f$-globulin). The human alpha$_1$-foetoprotein reaches a maximum concentration in 13-14 week foetuses, declines rapidly thereafter and usually disappears by the time of birth, but traces may be found during the first week of life (Gitlin and Boesman, 1966, 1967). This protein should be distinguished from the beta-foetoprotein in humans which is analogous to the alpha$_2$-foetospecific serum protein of rats (alpha$_2$-glycoprotein, alpha$_2$-macroglobulin, acute-phase protein, slow alpha$_2$-globulin); these proteins are not specific for hepatocellular carcinoma and are also associated with non-neoplastic toxic and regenerative states of the liver.

The purpose of this paper is to present the results of the alpha-foetoprotein test on cases of hepatocellular carcinoma and other selected diseases in Singapore. This investigation was part of a collaborative research programme that was co-ordinated and supported by the International Agency for Research on Cancer.

MATERIALS AND METHODS

Venous blood (5 to 10 cc.) was collected from 120 hospital patients comprising 33 cases of hepatocellular carcinoma, 2 cases of cholangiocellular carcinoma, 4 cases of secondary carcinoma of the liver, 3 cases of embryonal carcinoma of the testis, 24 other cancers (including 15 nasopharyngeal carcinomas), 25 cases of cirrhosis, 2 cases of hepatitis and 27 miscellaneous diseases comprising a wide variety of diseases.

The blood samples were collected between October 1967-September 1968. The serum was separated within four hours of collection, divided into ½-1 ml. aliquots, code-labelled and stored at —70°C. At the end of the collection period the serum aliquots were transported by air, packed in dry ice, to the laboratories in Moscow (Abelev) Astrakhan (Tatarinov) and Villejuif (Uriel). The alpha-foetoprotein assays were made by a standard Ouchterlony immunodiffusion method (Tatarinov, 1966; Abelev, 1967). The clinical and histological data on these cases were assembled in Singapore and submitted to the IARC in Lyon (O'Conor). This was a "double blind" investigation inasmuch as the serological and the clinical-pathological diagnoses were made independently.

RESULTS

The results of this investigation are presented in Table I. The three laboratories responsible for the alpha-foetoprotein assays produced identical results in 118 out of the 120 cases; the majority decision was accepted in the two cases where there was disagreement. The 33 cases of hepatocellular carcinoma in this series comprised 1 case (AFP+ve) in the 0-9 age group, 8 cases (5 AFP +ve) in the 20-49 age group, 23 cases (18 AFP +ve) in the 50-79 age group, and 1 case (AFP +ve) in

TABLE I

RESULTS OF THE ALPHA-FOETOPROTEIN TEST IN THE 120 CASES EXAMINED

Diagnosis	Number of Cases(*)		Alpha-foetoproteins	
			Positive	Negative
Hepatocellular Carcinoma	33	(30)	25 (76%)	8
Cholangiocellular Carcinoma	2	(2)	—	2
Secondary Carcinoma in the liver	4	(4)	—	4
Embryonal Carcinoma of the testis	3	(3)	2	1
Other Cancers	24	(23)	—	24
Cirrhosis	25	(21)	—	25
Hepatitis	2	(2)	—	2
Miscellaneous	27		—	27

* Numbers of histologically confirmed cases are given within brackets.

TABLE II

ALPHA-FOETOPROTEIN IN HEPATOCELLULAR CARCINOMAS

Country of Origin	Author(s)		Number of Patients	Positive Reaction	%
Uganda	Alpert *et al*	(1968)	40	20	50%
France	Uriel *et al*	(1969)	22	10	55%
Hong Kong	Smith and Todd	(1968)	32	19	59%
U. S. S. R.	Abelev *et al*	(1967)	28	17	60%
South Africa	Purves *et al*	(1968)	133	100	75%
Singapore	*This series*	(1969)	33	25	76%
Senegal	Uriel *et al*	(1968)	101	82	81%

which the age was not recorded; there were 23 males (17 AFP positive), 9 females (7 AFP +ve) and 1 case (AFP +ve) in which the sex was not recorded.

COMMENT

The proportion of hepatocellular carcinomas that are AFP positive has varied from 50 to 80% in different geographical areas (Table II). The report that hepatocellular carcinomas in rats induced by amino-azo dyes and nitrosamines are AFP positive, while those induced by aflatoxin are AFP negative (Stanislawski-Birencwajg *et al*, 1967) has led to some speculation that these geographical differences may reflect differences in the aetiology of liver cancer. It would be necessary, however, to extend these investigations by examining larger series of cases in order to exclude the possibility of random sampling fluctuations.

It is interesting to note that the proportion of AFP positive hepatocellular carcinomas in Singapore is relatively high (76%); the proportion in Hong Kong, the only other Asian population on which data is available, is 59%. The proportion of AFP positive liver cancers in Singapore does not appear to be influenced by age or sex. No clinical or histological difference has been found between AFP positive and AFP negative cases of hepatocellular carcinoma.

Another point of interest is that cholangiocellular carcinomas of the liver are AFP negative (Tatarinov and Nogaller, 1966; Abelev *et al*, 1967). This strengthens the view that hepatocellular and cholangiocellular carcinomas are heterogenous tumours (Shanmugaratnam, 1956). These neoplasms differ not only in their histological appearances but also in their epidemiology, aetiology and biological behaviour.

The AFP test has a remarkably high specificity for hepatocellular carcinoma. False positives are exceptionally rare. On the basis of all published data, patients with the presence of alpha-foetoprotein in the serum may be presumed to have liver cell carcinoma provided that neonatal jaundice and embryonal carcinoma are excluded. Two cases of so-called "false positive" AFP tests have been recorded (Alpert *et al*, 1968), but the possibility of an occult hepatocellular carcinoma has not been excluded in these cases. Consequently, while it is possible that some false positives may be discovered in future, it is reasonable to conclude that they would be exceedingly rare. The proportion of false negatives (20 to 50%, average 30%) may be reduced by employing the more sensitive radio-immunoassay techniques, but increasing the sensitivity greatly may well result in the appearance of false positives.

The alpha foetoprotein test is a relatively simple laboratory test by which some 75% of all hepatocellular carcinomas may be diagnosed without any risk to the patient. It is far superior to any liver function test, or combination of tests in the diagnosis of liver cancer and is almost as good as needle biopsy of the liver. The diagnosis of hepatocellular carcinoma would seldom be missed if both needle biopsy and the alpha-foetoprotein test are employed routinely.

SUMMARY

One hundred and twenty patients (33 cases of hepatocellular carcinoma, 3 cases of embryonal carcinoma of the testis and 84 controls) from Singapore were investigated for the presence of serum alpha-foetoprotein (AFP). The alpha-foetoprotein test was positive in 25 (76%) of the 33 cases of hepatocellular carcinoma, and in 2 of the 3 cases of embryonal carcinoma of the testis. All the 84 controls (cirrhosis 25 cases, hepatitis 2 cases, cholangiocellular carcinoma 2 cases, secondary liver carcinoma 4 cases, other cancers 24 cases, and miscellaneous diseases 27 cases) gave negative results. These results, which confirm the high specificity of these tests for hepatocellular carcinoma, are discussed in conjunction with reports in the literature.

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REFERENCES

1. Abelev, G.I., Perova, S.D., Khramkova, N.I., Postnikova, Z.A. and Irlin, I.S. (1963): "Production of embryonal a-globulin by transplantable mouse hepatomas," Transplantation, 1, 174.
2. Abelev, G.I., Assecritova, I.V., Kraevsky, N.A., Perova, S.D. and Perevodchikova, N.I. (1967): "Embryonal serum alpha-globulin in cancer patients: diagnostic value," Int. J. Cancer, 2, 551.

3. Alpert, M.E., Uriel, J. and De Nechaud, B. (1968): "Alpha Fetoglobulin in the Diagnosis of Human Hepatoma," The New England Journal of Medicine, 278, 984.
4. Bergstrand, C.G. and Czar, B. (1956): "Demonstration of new alphaprotein fraction in serum from the human fetus," Scand. J. Clin. Lab. Invest., 8, 174.
5. Gitlin, D. and Boesman, M. (1966): "Serum alpha-fetoprotein, albumin and gamma-G-globulin in the human conceptus," J. Clin. Invest. 45, 1826.
6. Gitlin, D. and Boesman, M. (1967): "Sites of serum alpha-fetoprotein synthesis in the human and in the rat," J. Clin. Invest. 46, 1010.
7. Masopust, J., Kithier, K., Radl, J., Kontecky, J. and Kotal, L. (1968): "Occurrence of fetoprotein in patients with neoplasms and non-neoplastic diseases," Int. J. Cancer, 3, 364.
8. Pedersen, K.O. (1944): "Fetuin, new globulin isolated from serum," Nature (London), 154, 575.
9. Purves, L.R., Macnab, M., Geddes, E.W. and Bersohn, I. (1968): "Serum-alpha-foetoprotein and primary hepatic cancer," Lancet, 1, 921.
10. Shanmugaratnam, K. (1956): "Primary carcinomas of the Liver and Biliary Tract," Brit. J. Cancer, 10, 232.
11. Smith, J.B. and Todd, D. (1968): "Foetoglobin and primary liver cancer," Lancet, 2, 833.
12. Stanislawski-Birencwajg, M., Uriel, J. and Grabar, P. (1967): "Association of Embryonic Antigens with Experimentally Induced Hepatic Lesions in the Rat," Cancer Res., 27, 1990.
13. Tatarinov, Yu.S. (1964): "Presence of embryospecific alpha-globulin in the serum of patients with primary hepatocellular carcinoma," Vopr. Med. Khim., 10, 90.
14. Tatarinov, Yu.S. (1966): "Content of embryospecific alpha globulin in fetal and neonatal sera and sera from adult humans with primary carcinoma of the liver," Fedn. Proc., 25, T344.
15. Tatarinov, Yu.S. and Nogaller, A.M. (1966): "Diagnostic importance of the embryo-specific a-globulin in the blood serum of hepatoma patients," Vopr. Onkol., 12, 26.
16. Uriel, J., de Nechaud, B., Stanislawski-Birencwajg. M., Masseyeff, R., Leblanc, L., Quenum, C., Loisillier, F. and Grabar, P. (1968): "Le Diagnostic du Cancer Primaire du Foie Par des Methodes Immunologiques," La Presse Medicale, 76, 1415.
17. Uriel, J. (1969): "Transitory liver antigens and primary hepatoma in men and rats," Pathologie et Biologie, 17, No. 8. In press.