ALPHA$_1$-FETOPROTEIN IN LIVER DISEASE IN SARAWAK

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SYNOPSIS

This is a preliminary report of 46 sera tested for Alpha$_1$-Fetoprotein (AFP) by the Counter immuno-electrophoresis technique in which biopsies for histopathology were also submitted. In 42 cases the needle biopsy of the liver was available. The material was divided into two groups on the basis of AFP positive and AFP negative sera and their histological diagnosis. The overall positivity rate in proven Primary liver cell carcinoma was 64.2 per cent.

INTRODUCTION

As the name indicates this fraction of proteins occurs in the serum of human faetus and persists for nearly three weeks during the post natal period. In the serum of healthy human adults it is not present. For the first time Tatarinov (1966) reported the presence of this fraction in the serum of two patients with Hepatocellular Carcinoma. Subsequently it was found to be associated with primary malignant neoplasms of the liver and rarely with gonadal and other malignant tumours in man.

During the last 10 years various method have been employed to detect this fraction in human serum. At present Radioimmune Assay is claimed to be the most specific and reliable method. This method, unfortunately, involves a lot of expenditure both on equipment and reagents, which only a few sophisticated laboratories can afford.

Anthony et al (1973) in their series have reported, "Over 70% of liver cell carcinomas were AFP positive in this series". Ackerman (1974) has stated that in areas where Hepatoma is endemic the positivity rate is as high as 75 per cent or more. Prakash et al (1976), in a study of 105 histologically proven cases of liver diseases, have reported their findings on Alpha$_1$-Fetoprotein (AFP) by the modified agar-gel diffusion technique of Prince (1968). They observed AFP positivity rate of 52.4 per cent in Primary liver cell Carcinoma, 3.5 per cent in Cirrhosis of liver and none with

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metastatic tumours in liver. Islam et al (1977) have reported a study of Primary liver cell Carcinoma with serum AFP test in 10 out of 17 cases, with a positivity rate of 58.8 per cent.

In Sarawak the incidence of histologically proven Primary Carcinoma of the liver is 5.85 per cent of all malignancies detected over a period of two years 1976 — 1977 (Kothare, 1978). It is, therefore, justifiable to carry out the test for Serum Alpha$_1$-Fetoprotein (AFP) to detect early Primary liver cell Carcinoma, thus enabling the Clinician to institute early Chemotherapy which may offer a longer span of life even though the ultimate prognosis will remain unchanged.

MATERIAL AND METHODS

Between May, 1977 and January, 1978, ninety-eight sera were tested for Alpha$_1$-Fetoprotein (AFP) by the Counter immuno-electrophoresis technique in the Central Laboratory, Kuching, Sarawak. These sera were referred from different hospitals in the State of Sarawak although the majority of samples received were from Sarawak General Hospital, Kuching. In a number of cases needle biopsy of liver was sent just before or within the next 4-5 days of receipt of the serum for AFP. Thus out of 46 patients with biopsy material, histopathology of liver was available in 43 cases. Cord blood of 8 newborns (Chinese 2, Dayaks 3, Malays 3) was tested and as expected was positive for AFP in all. Serum of 63 blood donors (Chinese 10, Dayaks 24, Malays 26, Others 3) was also tested for the same and all were negative. AFP positive sera were also tested for HB_sAg by Counter immuno-electrophoresis; all were negative.

The method for Counter immuno-electrophoresis adopted to suit the local conditions is described below. The reading was taken by one of the workers (BT) who was not aware of the histopathology report. Similarly the other worker (SNK) reported on the liver biopsy without any information regarding the AFP result of the particular patient, thus eliminating personal bias. The following procedure for Counter immuno-electrophoresis was done.

METHOD

COUNTER IMMUNO-ELECTROPHORESIS.

BUFFER: Diethylbarbiturate acetate PH 8.2 (ionic strength 0.1)

Sodium diethylbarbiturate	8.92 g.
Sodium acetate trihydrate	5.85 g.
Hydrochloric acid 0.1 N	140 ml.
Distilled water add to	1000 ml.

AGAROSE-GEL PREPARATION

Buffer solution was to be diluted 1:2 (50 ml. buffer plus 50 ml. distilled water). Prepare 1% agarose solution made from a commercial product. 10 ml. of the agar solution was used on a glass plate (10 cm. × 10 cm. size).

TECHNIQUE

Two rows of wells (diameter 3 mm.) were punched on the gel at a distance of 8 mm. apart (circumference to circumference). The positive control serum and the patients' sera were deposited toward the cathode and the Alpha$_1$-Fetoprotein antiserum, a commercial product, towards the anode. Electrophoresis was run for 45 minutes at a current of 1.5 — 2 MA per cm. The agar gel was kept in a wet chamber at room temperature to permit further antibody/antigen reaction for 1½ — 2 hours and was found to show a better and sharper line of precipitation, particularly in weakly positive sera.

The material is divided into two groups; Group I with positive AFP and Group II with negative AFP and their histopathology reports.

In group I there were 13 cases. (Table I) Amongst these there were 9 cases of proven Primary liver cell carcinoma (PLCC), 1 with probably metastatic carcinoma, 1 with Cirrhosis and 2 without liver biopsy. One of the latter was metastatic Embryonal cell carcinoma in inguinal lymph node in a previously proven case of a malignant precoccygeal Teratoma in a 2 months old baby.

In group II there were 33 cases, (Table II) amongst which 5 were with Primary liver cell carcinoma, 2 with Cholangiocarcinoma, 5 with metastatic carcinoma in the liver, 5 with Cirrhosis, 8 without any significant pathology, one each of Nodular hyperplasia of liver, Extrahepatic cholestasis, Chronic persistent hepatitis, Fatty change and diffuse necrosis of liver, 2 of Viral hepatitis and one with necrotic tissue debris from the liver site.

Reports of Liver function tests were available in 8 AFP positive cases, 7 of which were with PLCC and in 2 AFP negative with PLCC. In the AFP positive PLCC group 5 had unconjugated bilirubin between 1.0 — 2.3 mg per 100 ml.; in 2 cases the total proteins were below 6.0 g per 100 ml. and in all the albumin fraction was below 4.0 g. per 100 ml. with a proportionate rise of globulin

TABLE I
ALPHA$_1$-FETOPROTEIN POSITIVE (Group I)

ALPHA$_1$-FETOPROTEIN IN LIVER DISEASES			LIVER FUNCTION TEST								
SERIAL NO.	HISTOPATHOLOGY	ALPHA$_1$-FETO-PROTEIN	BILIRUBIN mg./100 ml.		PROTEIN g./100 ml.			ALKALINE PHOSPHA-TASE K.A. UNITS	TRANSAMINASES SIGMA-FRANKEL UNITS		THYMOL TURBIDITY UNITS
			Uncon j.	Con j.	TO-TAL	ALB.	GLOB.		SGOT	SGPT	
2/77	PRIMARY LIVER CELL CARCINOMA	POSITIVE	1.4	0.7	2.9	1.1	1.8	22.7	104	83	5
4/77	METASTATIC CARCINOMA LIVER	POSITIVE					NOT AVAILABLE				
10/77	EMBRYONAL CELL CARCINOMA (Inguinal Lymph node)	POSITIVE					NOT AVAILABLE				
20/77	EARLY CIRRHOSIS LIVER	POSITIVE	1.2	0.4	6.8	3.1	3.7	5.0	47	20	6
34/77	NO LIVER BIOPSY	POSITIVE					NOT AVAILABLE				
44/77	PRIMARY LIVER CELL CARCINOMA	POSITIVE	1.9	0.9	7.0	3.1	3.9	21	118	15	—
46/77	PRIMARY LIVER CELL CARCINOMA	POSITIVE					NOT AVAILABLE				
52/77	PRIMARY LIVER CELL CARCINOMA	POSITIVE					NOT AVAILABLE				
57/77	PRIMARY LIVER CELL CARCINOMA	POSITIVE	0.4	—	6.3	3.1	3.2	7.5	18	2	3
61/77	PRIMARY LIVER CELL CARCINOMA	POSITIVE	1.2	NOT DONE	7.6	4.0	3.6	19.5	94	19	3
64/77	PRIMARY LIVER CELL CARCINOMA	POSITIVE	1.0	0.7	5.8	2.4	3.4	27	185	61	—
66/77	PRIMARY LIVER CELL CARCINOMA	POSITIVE	2.3	0.7	7.0	2.9	4.1	24	61	17	—
72/77	PRIMARY LIVER CELL CARCINOMA	POSITIVE	0	0	6.5	3.1	3.4	15	26	12	—

TABLE II
ALPHA$_1$-FETOPROTEIN NEGATIVE (Group II)

ALPHA$_1$-FETOPROTEIN IN LIVER DISEASES

SERIAL NO.	HISTOPATHOLOGY	ALPHA$_1$-FETOPROTEIN
33/77	PRIMARY LIVER CELL CARCINOMA	NEGATIVE
40/77	SUGGESTIVE OF LIV. CEL. CARC.	NEGATIVE
56/77	CHOLANGIO-CARCINOMA	NEGATIVE
69/77	PRIMARY LIVER CELL CARCINOMA	NEGATIVE
20/78	PRIMARY LIVER CELL CARCINOMA	NEGATIVE
16/78	LIVER CELL CARCINOMA ASSOCIATED WITH CIRRHOSIS	NEGATIVE
1/77	METASTATIC CARCINOMA, LIVER	NEGATIVE
6/77	NECROTIC TUMOUR TISSUE	NEGATIVE
9/77	METASTATIC ADENOCARCINOMA	NEGATIVE
11/77	VIRAL HEPATITIS	NEGATIVE
12/77	PORTAL CIRRHOSIS	NEGATIVE
19/77	METASTATIC ADENOCARCINOMA	NEGATIVE
23/77	METASTATIC ADENOCARCINOMA	NEGATIVE
24/77	METASTATIC ADENOCARCINOMA	NEGATIVE
27/77	SUGGESTIVE OF HEPATITIS	NEGATIVE
30/77	CIRRHOSIS WITH FATTY CHANGE	NEGATIVE
47/77	CIRRHOTIC CHANGE	NEGATIVE
48/77	NO EVIDENCE OF NEOPLASM	NEGATIVE
53/77	CHOLANGIO-CARCINOMA	NEGATIVE
54/77	NO EVIDENCE OF NEOPLASM	NEGATIVE
55/77	DIFFUSE NECROSIS OF LIVER	NEGATIVE
59/77	NO SIGNIFICANT PATHOLOGY	NEGATIVE
62/77	NO EVIDENCE OF NEOPLASM	NEGATIVE
65/77	NODULAR HYPERPLASIA LIVER	NEGATIVE
68/77	CHOLESTATIC JAUNDICE PROBABLY OF EXTRAHEPATIC ORIGIN	NEGATIVE
77/77	NO SIGNIFICANT PATHOLOGY	NEGATIVE
78/77	CHRONIC PERSISTENT HEPATITIS	NEGATIVE
8/78	FATTY CHANGE	NEGATIVE
14/78	PORTAL CIRRHOSIS	NEGATIVE
15/78	NO SIGNIFICANT PATHOLOGY	NEGATIVE
17/78	NO SIGNIFICANT PATHOLOGY	NEGATIVE
21/78	NO SIGNIFICANT PATHOLOGY	NEGATIVE
22/78	EARLY CIRRHOSIS LIVER	NEGATIVE

fraction. The S.G.O.T. was increased in 6 and ranged between 47 — 185, while the S.G.P.T. was raised in 2 cases with 61 and 83 Sigma-Frankel units per ml. The Serum alkaline phosphatase was increased in 6 cases and ranged between 15 — 27 K.A. units per mg phenol per 100 ml.

In 2 AFP negative PLCC cases the total proteins were normal with slight diminution of albumin fraction. The Serum Alkaline phosphatase was raised in both and the S.G.O.T. and S.G.P.T. were markedly raised in one.

DISCUSSION

When the first group was considered there were 9 histologically proven cases of PLCC out of 11 cases. If proven cases of PLCC in both groups were considered the positivity rate is 64.2 per cent. If cases of liver malignancies, including metastatic cancers in both groups, were considered the positivity rate falls to 40.9 per cent.

It was interesting to note that in the only case of Embryonal Cell Carcinoma AFP was detected in the serum, which though rare is known to occur. This could be an expression of continued faetal type of liver activity. However, the possibility of hepatic metastases inducing AFP production cannot be ruled out. As such tumours are rare, accumulated data from various sources, supported preferably by autopsy findings, would be helpful in understanding the pathogenesis of this finding.

One case of AFP positive and histologically diagnosed as "Metastatic Carcinoma" in the liver on the Needle biopsy may, in fact, prove to be a Liver Cell Carcinoma. In such cases, therefore, positive AFP test would justify the need to either review or repeat the liver biopsy.

There was one AFP positive case of "Liver Cell Carcinoma on Cirrhotic liver and another of "Early Cirrhosis". The positive AFP test in the latter was suggestive of a malignant transformation in other parts of the liver, justifying repeat liver biopsy or further investigations to exclude PLCC.

Anthony et al (loc cit) investigated serum AFP in 134 out of 552 cases of Cirrhosis of liver, Cirrhosis with liver cell dysplasia, Cirrhosis with liver cell Carcinoma and dysplasia. In only 14 out of 64 cases of Cirrhosis with dysplasia and in 32 out of 45 cases of Cirrhosis with Carcinoma and associated dysplasia, the AFP test was positive. They concluded that AFP test is negative in Cirrhosis with cell dysplasia and hence cell dysplasia by itself is not an indicator of malignant transformation.

Liver function tests in the present series were abnormal as expected and the range varied considerably; this probably depended upon the pre-existing liver disease and the extent of malignant tissue.

In 1975 in all hospitals of Sarawak, 203 cases of Cirrhosis of liver were recorded in a total of 52,621 in-patients excluding diseases due to traumatic and unnatural causes, with an incidence of 0.38 per cent. (Annual Report, Medical and Health Department, Sarawak, 1975, page 152). In Sarawak General Hospital, Kuching, 259 cases of Cirrhosis were recorded in a total of 23,445 in-patients excluding diseases due to traumatic and unnatural causes, during the year 1976 (Annual Report: Bahagian Pertama, Sarawak, Malaysia; Medical and Health department 1976, page 93), with an incidence of 1.1 per cent of all admissions. It would be pertinent to undertake a long term study in such cases with repeated AFP test and liver biopsy to confirm the findings of Anthony et al (loc cit) and also to estimate the incidence of malignant transformation in Cirrhotic liver in a country where Primary liver cell carcinoma is endemic.

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