α₁-FETOPROTEIN AND CARCINOEMBRYONIC ANTIGEN IN A PATIENT WITH CARCINOID OF THE STOMACH

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A case of carcinoid of the stomach with metastases to the liver is described in which the simultaneous occurrence of α₁-fetoprotein (110 μg/ml) and carcinoembryonic antigen (25 ng/ml) was demonstrated in the circulation by immunochemical methods. By means of the indirect fluorescence method α₁-fetoprotein was detected in the cytoplasm of some tumorous cells both in the primary tumour and in liver metastases. On the other hand, the abnormal variant of alkaline phosphatase isoenzymes — the Regan isoenzyme — was not detected in the patient’s serum though the total activity of alkaline phosphatase in serum was increased (150 U/L). The patient’s serum also contained “hepatitis B-associated” antigen.

This case provides a further evidence of another pathologic occurrence of α₁-fetoprotein in tumours of the digestive tract; it also shows the possibility of the simultaneous incidence of two “onco-fetal antigens” in one type of the tumour.

The immunoprecipitation test detecting α₁-fetoprotein proved to be a very useful marker of hepatocellular carcinoma and some teratoblastomas.1,13,16,30 On the basis of results of studies performed on larger groups of patients the high specificity of the test was generally assumed.3,12,18,21,31 In recent literature, however, some papers have appeared describing the occurrence of α₁-fetoprotein in cases of gastric carcinoma with metastases to the liver,3,6,9,15,19 and in viral hepatitis of infants17 and adults.9 By means of other techniques such as immunoaautoradiography, aggregate hemagglutination,2 and sandwich-counter-immunoelectrophoresis,25 a higher sensitivity of tests was achieved. On the other hand, the percentage of “false-positive” cases increased.2

By means of radioimmunoassay, α₁-fetoprotein was demonstrated in nanogram amounts even in sera of healthy adult persons.23,24 Higher concentration of α₁-fetoprotein (of the order of mg/L), however, occurs above all in primary hepatomas and in some teratocarcinomas. The occurrence of α₁-fetoprotein detectable by the conventional immunodiffusion method in tumours other than those mentioned above is interesting with regard to the possible site of α₁-fetoprotein synthesis. The question arises whether α₁-fetoprotein can be synthesized by tumorous cells themselves or whether the α₁-fetoprotein synthesis is induced by metastases into the liver.

In this paper we present a case of gastric carcinoid with metastases to the liver in which a high concentration of α₁-fetoprotein in blood serum was demonstrated. Preliminary report on this case was published earlier.33

**MATERIALS AND METHODS**

*Case Report*

The patient K.F. (No. 13689/71), a 54-year-old man, was admitted to the 1st Clinic of Interanl Medicine, Charles University Faculty Hospital, Prague, with the diagnosis of suspected liver fibrosis and chronic pancreatitis in acute exacerbation. His complaints lasting 6 months began with epigastric pain, nausea, malaise, then jaundice, anorexia, and weight loss (6 kg). On physical examination icterus of sclerae and skin was found; the abdomen on palpation was soft, the liver enlarged (2 1/2 finger breadths below the

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costal margin), on palpation soft and painless. The erythrocyte sedimentation rate was increased (reaching 103 mm/hr and 130 mm/2 hr.)

**Biochemical Examination** revealed markedly increased level of bilirubin (14~19.5 mg/100 ml), alkaline phosphatase (40 to 64 K.A.U.), transaminases (SGOT 62-130 and SGPT 29-70 Reitman-Frankel units), total lipids (1,640~2,160 mg/100 ml), cholesterol (496~550 mg/100 ml), \( \beta \)-lipoprotein (940 mg/100 ml), and mucoproteins (7.5 mg/100 ml of tyrosine). The electrophoretic pattern of serum proteins was also changed (albumin 3.13, \( \alpha \)-globulins 1.86, \( \beta \)-globulins 1.00, and \( \gamma \)-globulin 0.57 g/100 ml); immunoglobulins (IgG and IgM lowered to 880 and 50 mg%, respectively, and IgA increased to 560 mg%). Other biochemical parameters (electrolytes, urea, creatinine) were within normal limits. Urine was strongly positive for bilirubin.

There was the normocytic anaemia in the blood picture (erythrocyte count 3.2~3.9 millions/mm³, haemoglobin 8.6 to 10.9g/100ml) with reticulocytosis 33-52/mille. The leucocyte count was slightly increased (8,200~10,300/mm³) with relative lymphopenia (2~6%). The blood group was A1Rh+.

**X-Ray examination** disclosed extensive tumorous infiltration of aboral two-thirds of the stomach with impaired evacuation of contrasting material.

The tumour was not suitable for radical surgical treatment. The patient was treated symptomatically with analgesics, infusions of fructose and vitamins, and with blood transfusions. Cyclophosphamide was also administered. The disease progressively developed, icterus deepened, ascites appeared, and the patient died within 3 weeks in tumorous cachexia.

**Clinical Diagnosis** Gastric carcinoma with metastases to the liver, secondary obstructive icterus caused probably by the tumorous blockade of bile ducts, secondary anaemia.

**Postmortem Examination** An extensive flat tumour measuring 8 cm was localized on the small curvature of the stomach. The tumour occupied the entire wall, had yellow colour, and was considerably necrotic. Extensive metastases to the liver measuring as much as 9 cm grew through up to the duodenum, infiltrated the choledochus wall, and caused its stenosis.

**Microscopic Examination** The tumour consisted of epithelial masses and revealed an alveolar pattern in some places. Some of the cell nuclei were atypical. The cytoplasm contained sporadically oxyphilic granules (Photo 1a). These granules were argyrophilic in Bodian’s reaction (Photo 1a). These granules were argyrophilic in Bodian’s reaction (Photo 1b). Liver metastases showed the same picture.

**Histochmical Examination** Grimelius reaction was weakly positive, masked metachromasia (Solcia) was not remarkable, and diazo reaction was negative.

**Electron-microscopic Examination** Ultrathin sections were prepared from specimens previously embedded in paraffin. The material was postfixed with OsO₄. The sections were contrasted with uranyl acetate and lead citrate (Reynolds). In a number of tumour cells granules similar to the ECL type were demonstrated, some cells contained isolated round-shaped granules resembling A granules, and one biconcave granule of the EC type was found only in one cell.26)

**Conclusion** Carcinoid of the stomach non-producing serotonin, with metastases to the liver.

**RESULTS**

**Immunochernical Examination**

**Detection of \( \alpha_1 \)-Fetoprotein** An antigen identical with that in fetal serum was detected by means of immunoprecipitation on Ouchterlony plates using the specific goat antiserum and rabbit antiserum against human \( \alpha_1 \)-fetoprotein antigen (Fig. 1). This antigen moved on immuno-electrophoresis in the region of \( \alpha_1 \)-globulins and gave also the reaction of antigenic identity with \( \alpha_1 \)-fetoprotein in human fetal serum (Fig. 2). The concentration of \( \alpha_1 \)-fetoprotein determined by the technique of radial immunodiffusion equalled to 110 mg/L.

**Detection of \( \alpha_2 \)-Fetoprotein and \( \beta \)-Fetoprotein** The patient’s serum did not give the precipitation lines on Ouchterlony plate either with the antiserum against \( \alpha_2 \)-fetoprotein or with the antiserum against \( \beta \)-fetoprotein.

**Detection of “Hepatitis B-associated” antigen (HB-Ag)** HB-antigen was demonstrated in the patient’s serum by means of counter-immunoelectrophoresis (Austigen-test, Hyland).

**Detection of Abnormal Isoenzymes of Alkaline Phosphatases (the Regan Isoenzyme)** Agar gel electrophoresis, inactivation-inhibition method, as well as heat-inactivation method, failed to prove the presence of alkaline phosphatase of placental type (the Regan isoenzyme) or of any other abnormal variant in the patient’s serum. The markedly increased total activity of serum alkaline phosphatase (150 U/L) resulted from an increased level of the liver isoenzyme.
Detection of Carcino-embryonic Antigen (CEA) in Serum

CEA in serum samples was demonstrated by the indirect microradioimmunochemical method\(^{14}\); its concentration reached 25 µg/L. This concentration is strongly indicative of disseminated cancer.

Immunohistological Examination

Detection of \(\alpha_1\)-fetoprotein was performed by the indirect immunofluorescence technique in paraffin sections after fixation of blocks in formol according to Purtilo et al.\(^ {22}\) and Engelhardt et al.\(^ {8}\). After deparaffination, the sections were washed three times in physiological saline (2 hr) and incubated for 30 min with diluted rabbit anti-\(\alpha_1\)-fetoprotein serum (Boehringerwerke, Frankfurt/Main) (1 part of antiserum and 12 parts of saline). After incubation the sections were washed 10 times in saline. They were then fixed with ethanol (10–20”) and again washed three times with saline. Thereafter, they were covered with a layer of goat anti-rabbit Ig serum labelled with fluoresceine isothiocyanate (GAR/FITS Sevac, Prague). Before using, the conjugated antiserum was purified by column chromatography on Sephadex G-25 and absorbed with acetone powder from the rabbit and human liver. Incubation lasting 30 min was performed in a large dark chamber. After incubation, the preparations were repeatedly rinsed with physiologic saline, fixed with ethanol for a short period, again washed, and mounted in buffered glycerol. The preparations were examined with a fluorescence microscope ML-2 (U.S.S.R.) using the mercury lamp HBO-200, and the excitation blue filter BG-12 and the yellow closing filter 2. Sections from a non-tumorous tissue of the same patient treated in the same way were used as controls. Another control were sections from the tumorous tissue treated in the same way except that the specific anti-\(\alpha_1\)-fetoprotein serum was absorbed with human fetal serum in advance. Positive fluorescence of the GAR-FITS-RAHu-\(\alpha_1\)-fetoprotein system detecting the presence of \(\alpha_1\)-fetoprotein was found only in some cells of the primary tumour in the stomach. The preparation obtained from the duodenum affected by the tumorous process growing through from liver metastases the specific fluorescence was apparent only in single tumorous cells. The specific fluorescence was diffusely localized in the whole cytoplasm, while the nuclei remained dark. The intensity of specific fluorescence was not uniform. Control preparations
did not show any fluorescence (with the exception of usual autofluorescence of the connective tissue).

**DISCUSSION**

The illness of the patient K.F. is interesting for several reasons. It is a case of carcinoid with the simultaneous presence of carcinoembryonic antigen (25 ng/ml) and \( \alpha \)-fetoprotein (110 \( \mu \)g/ml) in serum. An increased level of carcinoembryonic antigen in the patient is not surprising with regard to the fact that the positive finding of CEA in carcinomas of the digestive tract is frequent.\(^{28}\) On the other hand, the presence of \( \alpha \)-fetoprotein in malignant tumours other than hepatocellular carcinoma or teratocarcinoma is relatively rare. \( \alpha \)-Fetoprotein was also found in single cases of gastric adenocarcinoma,\(^{3,5-7,9,20}\) in gastric adenocarcinomas associated with prostatic carcinoma,\(^{15,16}\) and in the squamous cell carcinoma of the oesophagus.\(^{27}\)

The origin of \( \alpha \)-fetoprotein in those “atypical” fetoprotein-positive tumours could be of two kinds: (1) Synthesis in specific cells of the proper tumour or its metastases, and (2) synthesis in liver cells induced by the section of the primary tumour (“derepression” of the gene responsible for \( \alpha \)-fetoprotein synthesis in normal hepatocytes). The results of the immunohistological investigation in our study suggest the first of these possibilities. We could detect \( \alpha \)-fetoprotein in some tumorous cells only (both in the primary tumour of the stomach and in its metastases).

The main site of \( \alpha \)-fetoprotein synthesis in man and other mammals was proved to be in parenchymatous cells of the fetal liver\(^{10,22}\) and in some cells of the yolk sac.\(^{10}\) A trace synthesis of \( \alpha \)-fetoprotein was also demonstrated in some cells of the primitive gut.\(^{11}\) This finding is a possible explanation of the occurrence of \( \alpha \)-fetoprotein in rare cases of carcinoma of the digestive tract. We assume that small islets of one out of the three types of embryonal and fetal elements normally synthesizing \( \alpha \)-fetoprotein in ontogenesis persists in the organism even after birth. In case of their malignant proliferation the number of these fetoprotein-producing cells increases to such an extent that the level of \( \alpha \)-fetoprotein can be demonstrated by usual immunoprecipitation methods. In our opinion an increased \( \alpha \)-fetoprotein concentration can be rather attributed to the malignant proliferation of the cells originating from the elements producing physiologically \( \alpha \)-fetoprotein in the early stage of ontogenesis than to the adult cells in which the derepression of the gene responsible for \( \alpha \)-fetoprotein synthesis took place.

The negative finding of the specific fraction of alkaline phosphatase (the Regan isoenzyme) accompanied by a high activity of normal liver isoenzymes of alkaline phosphatase also supports our view assuming that the participation of liver is nonspecific and the source of \( \alpha \)-fetoprotein in this case is tumorous cells themselves. The finding of hepatitis B-associated antigen in our patient can be regarded as a concomitant phenomenon.

The immunohistological localization of \( \alpha \)-fetoprotein only in some tumorous cells not only suggests the biochemical heterogeneity of tumorous tissue but may also explain the possible morphological variability of extrahepatic fetoprotein-producing tumours (adenocarcinoma, squamous-cell carcinoma, carcinoid, etc.). The biochemical heterogeneity is also confirmed by the simultaneous occurrence of two onco-fetal antigens, namely, \( \alpha \)-fetoprotein and carcinoembryonic antigen.

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EXPLANATION OF PLATE

Photo 1. (a) Tumour metastases in the duodenum. Stained with Haematoxylin-Eosin. ×400. (b) Parallel section stained with Bodian’s technique. Numerous positively reacting granules are apparent in the cytoplasm of tumorous cells. ×600.

Photo 2. Tumorous infiltration of the gastric wall. Stained with Haematoxylin-Eosin. ×300.