The Clinical Significance of Human Alpha-fetoprotein

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ABSTRACT

Deviations from the normal of alpha-fetoprotein (AFP) concentrations in fetal serum, amniotic fluid, maternal serum and adult human serum can be explained by understanding the normal physiology and the pathophysiology of AFP synthesis and metabolism. AFP is the prototype of oncofetal markers. Emphasis is given to the usefulness of elevated serum AFP levels in the diagnosis and management of primary hepatomas and tumors of germ cell origin. The ability to detect neural tube defects early in gestation by monitoring maternal serum and amniotic fluid AFP concentrations is discussed.

Introduction

Human alpha-fetoprotein (AFP), whose existence was discovered 21 years ago, is the homologue of a serum protein found in all mammalian species during embryonic development. During the same interval, increasing attention has been paid by basic and clinical scientists to biochemical and immunological similarities between fetal development and neoplastic states in man and laboratory mammals. The study of so-called onco-fetal antigens began in 1963 when Abelev, working in the Soviet Union, described the re-emergence of murine AFP in the blood of mice bearing transplantable hepatomas. One year later, another Russian worker, Tatarinov, described similar findings in human patients with primary liver carcinomas. These observations, along with the description by Gold of carcinoembryonic antigen in cancers of the large bowel, thrust to the forefront the general topic of carcino-fetal antigens in the study and management of neoplastic diseases in man.

Continuing attention by a large number of investigators to the physiology and pathophysiology of AFP in fetal life has laid the groundwork upon which its aberrant emergence can be contemplated and understood, not only in hepatomas, but in tumors of germ cell origin and, less frequently, in tumors of the stomach and pancreas. In addition, it has been learned

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that elevated AFP levels may be encountered in the course of non-neoplastic diseases of the liver, as well as in such congenital or genetically determined diseases as ataxia telangiectasia and tyrosinemia.\textsuperscript{2,17,19,24} Finally, monitoring of AFP levels in maternal blood and fetal amniotic fluid during the course of pregnancy makes it possible to detect certain serious fetal diseases and malformations, most notably neural tube defects, early enough in gestation so that the choice of elective termination of the pregnancy can be offered to the parents.\textsuperscript{5,23}

Human AFP may be isolated from serum or ascitic fluid by classical techniques of protein purification, but trace contamination by serum albumin and other \(\alpha\)-globulins is difficult to eliminate. Large scale isolation by means of immuno-adsorbent techniques yields highly purified AFP preparations capable of being crystallized.\textsuperscript{9,19} The protein has a molecular weight of 67,500 daltons and is a single-chain sialylated glycoprotein containing approximately 3.6 percent carbohydrate. It is a negatively charged protein with an isoelectric point of pH 4.57 and displays several charge isomers by extended agarose gel electrophoresis, as well as by isoelectric focusing in acrylamide gels.\textsuperscript{13,14,25} These variations in charge, due only in part to variations in sialic acid content, do not affect the antigenic properties of the protein and present no hazard when quantitation by immunological techniques is contemplated. In addition, no antigenic differences have been identified between AFP's isolated from tumor bearing adults and from fetal sources.\textsuperscript{18,19}

Synthesis of AFP

Synthesis of AFP by the human embryo can be detected as early as 29 days after conception. At this stage of gestation, fetal AFP production is only partly due to synthesis by hepatocytes, since at least as much AFP synthesis can be found in the yolk sac, which is a well-defined vesicle attached to the foregut by a narrow stalk. In the human this organ becomes atretic by 11 to 12 weeks of gestation, and thereafter AFP synthesis occurs predominantly in fetal hepatocytes, although a small amount may be produced by the fetal gut. Between the seventh and tenth week of gestation, fetal serum AFP levels increase 30-fold from 70 to 2000 \(\mu\)g per ml; AFP levels reach a maximum of 3000 \(\mu\)g per ml at about the 14th week of gestation (figure 1), exceeding in its concentration all other fetal plasma proteins including albumin. From the 14th to the 32nd week of pregnancy fetal AFP serum concentrations decline exponentially with a half-life of approximately 32 days due largely to the dilutional effects of a rapid increase in fetal weight. From 32 weeks gestation, at which time fetal AFP serum concentration is 200 to 300 \(\mu\)g per ml, to term, an even more precipitous decline in AFP levels occurs (figure 2), related largely to a decrease in the rate of AFP synthesis, so that at term fetal levels have declined to 20 to 120 \(\mu\)g per ml.\textsuperscript{7,17}
Concentrations of AFP in Serum

During the first two months of life, AFP levels are approximately 400 ng per ml of serum. By six months they have fallen to 30 ng per ml, and by one to two years of age they have fallen to levels below 15 ng per ml.15 Thereafter and for the remainder of childhood and adult life, AFP levels are in the range of 3 to 15 ng per ml. That these low levels, detectable by radioimmunoassay, do in fact represent continued low level synthesis of AFP, which has a biological half-life of approximately six days, has been verified by the isolation of authentic AFP from a large pool of adult human serum by immunoadsorbent techniques.19

A variety of immunological techniques for the detection and quantitation of AFP have been utilized which vary greatly in their sensitivity. Relatively high levels of AFP (500 to 1500 ng per ml) may be quantitated by single radial immunodiffusion or rocket immunoelectrophoresis. The most widely utilized technique for the detection and quantitation of AFP levels between 5 and 500 ng per ml is the radioimmunoassay, although similar results can be obtained by rocket immunoelectrophoresis utilizing radioautography or peroxidase-linked staining techniques for amplification and development of the immune precipitate.5,17

The incidence of elevated AFP serum levels (>25 ng per ml) in primary hepatomas varies from one geographical area to the next and ranges from 50 to 90 percent; in this country the lower figure may be a more accurate estimate, while in Japan the higher figure prevails.2,17 Since approximately 10 to 20 percent of patients with non-malignant liver disease of all types have elevated serum AFP levels, criteria are needed to discriminate between the two groups. In general the degree of elevation may serve as an important distinction, since most patients with non-malignant liver disease have serum AFP levels below 500 ng per ml. Indeed, a serum AFP concentration above this indicates the presence of primary liver cancer 97 percent of the time.19 Elevated serum AFP levels in non-malignant liver disease tend to be fluctuant and/or transient, and serial determinations over a several month period can usually resolve doubtful cases; a steady or rising serum AFP level is more likely to be indicative of primary liver cell cancer. In large scale screening of a group of cirrhotic patients at higher than normal risk for hepatoma, approximately 22 percent of the group displayed elevated serum AFP levels; of these, about 10 percent were ultimately diagnosed as having hepatomas.12 A similar study in a high hepatoma-incidence area in Japan, where 4600 adults were subjected to serum AFP level screening, resulted in the detection and probable surgical cure of at least one case of hepatoma.10

In those instances where surgical resection of the tumor is attempted, postoperative serial AFP determinations will show an exponential fall to normal levels when the tumor has been completely excised. Recurrence of elevated AFP levels will almost certainly mean tumor recurrence. Unfortunately, since the chemotherapeutic treatment of hepatomas is at present largely unsuccessful, serial AFP levels will probably contribute little to the management of pa-
tients undergoing drug therapy, although newer, more effective drugs may eventually make this an important laboratory adjunct to the follow-up care of such patients.

In understanding the significance of elevated AFP levels in patients with germ cell tumors of the ovary, testis, or extra-gonadal primary sites, the classification of such tumors, according to Teilmann, is of the utmost importance, since it emphasizes the role of the yolk sac or endodermal sinus components of such tumors in relationship to AFP synthesis. The following points deserve special emphasis (figure 3):

1. Elevations of serum AFP levels are not seen in conjunction with pure seminomas of the testis or dysgerminomas of the ovary.

2. In mixed germ cell tumors (embryonal cell carcinomas), elevations of both AFP and the β-chain of human chorionic gonadotropin (HCG) may be seen singly or together. In those instances where serum AFP levels are elevated, careful histopathological examination of the tumor will usually reveal yolk sac elements, while HCG elevations are associated with the presence of syncytotrophoblastic tumor elements.

3. In pure tumors of extraembryonic origin derived from yolk sac elements (endodermal sinus tumors), AFP elevations are always present, and HCG is absent. In pure choriocarcinomas, the converse is true.

4. In teratoblastomas, AFP and HCG are usually absent; increased levels of either marker may, however, be present in those tumors which possess small areas of extraembryonic tumor tissue.

5. When such tumors are suspected, serum monitoring for both AFP and HCG should begin prior to surgery. The complete disappearance of elevated AFP levels, with an exponential fall of five to six day half-life, is a good indication of complete extirpation of such malignancies.

6. In surgically unresectable or metastatic disease, where a high proportion of such patients are now amenable to efficacious and possible definitive treatment by combination chemotherapy, long term serial follow up of both AFP and HCG serum levels can be undertaken.

7. In both surgically and medically treated patients, the reappearance of elevated serum levels of either AFP or HCG almost certainly heralds a clinical relapse, and appropriate diagnostic and therapeutic measures should be instituted. Chemotherapeutic intervention may then be started while the tumor mass is small and more easily eradicated.

Concentrations of AFP in Amniotic Fluid

Important information concerning fetal health can be derived from a study of AFP concentrations in fetal amniotic fluid and maternal serum during the course of gestation. The following de-
scription of the factors controlling AFP levels in these two compartments will aid in understanding pathophysiological changes.7,20

Greater than 98 percent of amniotic fluid protein is maternally derived. Probably the most important source of amniotic fluid AFP is its excretion into the fluid through the fetal urine. Accordingly, amniotic fluid AFP is highest at approximately 14 weeks of gestation, when fetal serum AFP levels are at their peak (figure 4). During the first trimester of gestation, amniotic fluid is an important source of AFP entering the maternal circulation by diffusion. Under normal circumstances, maternal serum AFP is increasingly derived from placental back diffusion; as pregnancy proceeds, the placenta enlarges, and placental permeability increases. Fetal swallowing of amniotic fluid, followed by AFP absorption or breakdown, is an important route of egress of AFP from the amniotic fluid.

Thus amniotic fluid AFP levels are highest during the 14th to 18th week of gestation, ranging from 5 to 35 µg per ml with a median value of approximately 15 to 20 µg per ml. They fall exponentially throughout pregnancy in parallel with the fall in fetal serum AFP levels. At term, amniotic fluid AFP levels range from 30 to 500 ng per ml, with a median of 100 to 200 ng per ml.17,20

Maternal serum AFP levels, presumed to be derived largely from the fetus, rise progressively throughout pregnancy, reaching a maximum at 32 to 34 weeks gestation (figure 5). At 16 to 18 weeks of gestation, the median maternal serum AFP level is approximately 50 ng per ml, rising to 100 to 300 ng per ml at peak levels later in gestation. In normal circumstances, maternal serum AFP levels at term can be correlated with placental size while amniotic fluid AFP levels are an
excellent indication of fetal gestational age.20

Among the pathological conditions of fetal development17,20 which have been associated with elevated amniotic fluid AFP levels are: (1) multiple pregnancy, (2) congenital nephrosis, (3) intrauterine fetal death, (4) hydrocephalus, (5) hemolytic disease of the newborn secondary to Rh isoimmunization, (6) omphalocele, (7) duodenal or esophageal atresia and (8) open neural tube defects (i.e., anencephaly, meningocele, myelocle).

Elevations of maternal serum AFP levels may also be seen in certain of the fetal conditions listed and are also found in conjunction with threatened and missed abortion and placental separation.20

Neural tube defects (NTD) represent one of the more common serious congenital malformations. The incidence of this malformation in the United States is 2 per 1000 births, while in England it is 4 per 1000 and in Northern Ireland 8 per 1000. The risk rises precipitously in those families with a prior history of NTD in parents or siblings. Prenatal detection of this anomaly, in those instances where the malformation is open or covered only by a thin membrane, is almost always possible by means of amniocentesis and determination of amniotic fluid AFP concentration, providing that contamination with fetal blood is excluded or appropriate correction made for its presence. In anencephaly, for example, the mean amniotic fluid concentration is 10 to 50 standard deviations above the normal mean, while in spinal cord NTD the levels are somewhat lower but easily differentiated from normal.

A recent study completed in England demonstrates the feasibility of using measurement of maternal serum AFP levels between 16 and 18 weeks of gestation as a screening test for selection of those women who should undergo amniocentesis for NTD detection.20 By selecting a maternal serum AFP level at 16 to 18 weeks gestation 2.5 times above the median as a cutoff, and excluding multiple pregnancy by ultrasonography, British women, who subsequently may be advised to undergo amniocentesis, have a risk of 1 in 10 of bearing a child with NTD to term. The British government plans to institute maternal serum AFP level determinations on a routine nationwide basis in April, 1978.3 It is ironic that in our own country reagents which would make similar screening possible have not yet been approved for commercial distribution by that withered arm of Big Brother, the F.D.A.16

References


