# A CORRELATIVE STUDY OF: ALPHA FETO PROTEIN AND SERUM ALKALINE PHOSPHATASE IN MALIGNANT LIVER DISEASES



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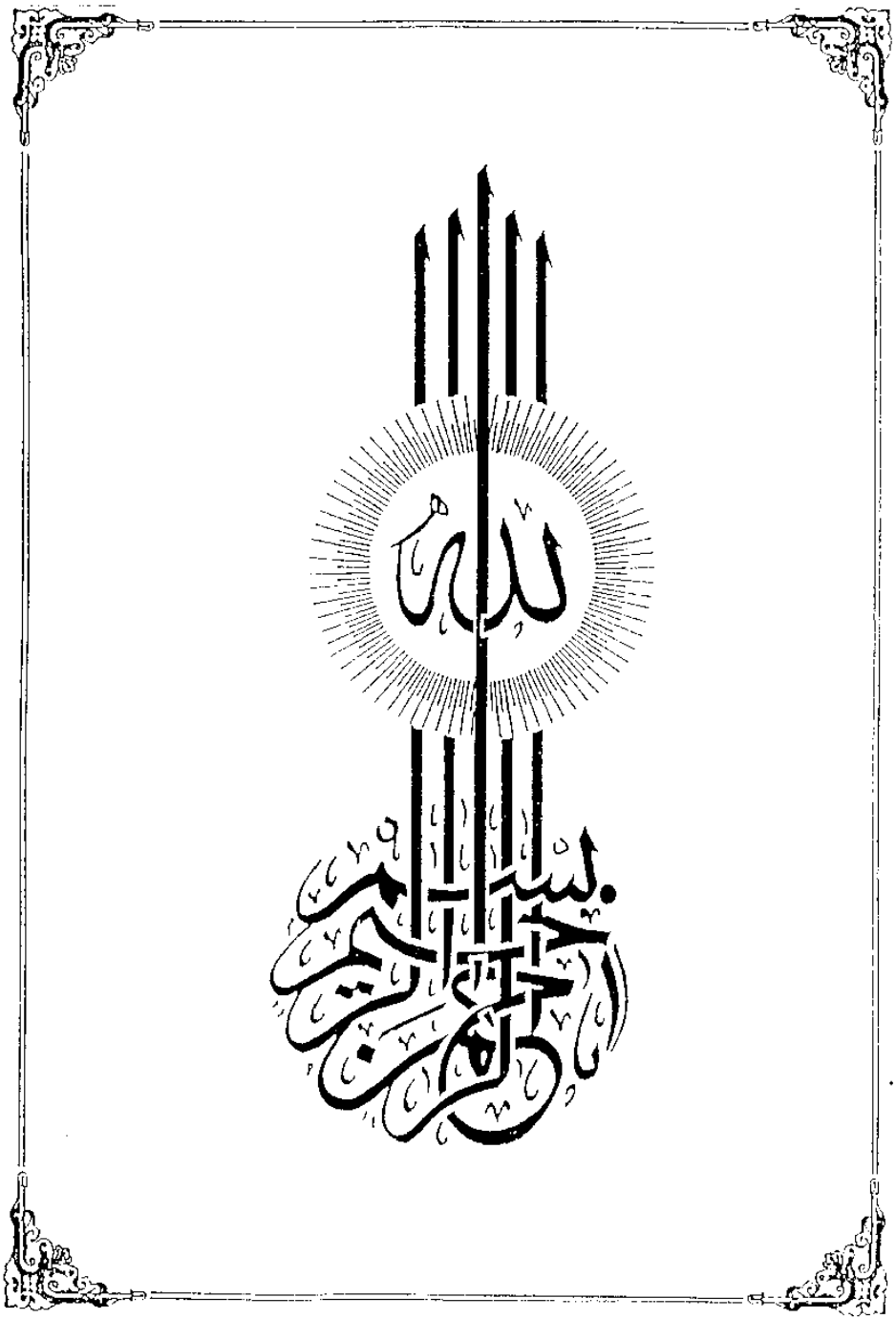

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# *Introduction*

# INTRODUCTION

Alpha fetoprotein (AFP) is a natural component of plasma protein in human foetus older than six weeks and reaches maximum concentration between 12 and 16 weeks. Few weeks after birth it disappears from the circulation but reappears in the blood of patients with primary liver cancer.

AFP is also present in the serum of patients with embryonic tumours of the ovary and embryonic hepatoblastoma. It may also be present with carcinoma of the gastrointestinal tract with hepatic secondaries.

This rise of AFP in the serum of human patients might provide a precise diagnostic test for liver carcinoma.

The significance of an elevated serum AFP concentration in the diagnosis of hepatocellular carinoma was soon well documented (Wepsic, 1979).

Increased level > 40 ng/ml of AFP have been demonstrated in a variety of non-neoplastic hepatic disorders. The serum concentrations of AFP are more frequently increased in those disorders in which there are prominent hepatocellular necrosis

and highest values were observed in patients with viral hepatitis (Bloomer et al., 1975).

There are three main aims in this study. First, is to estimate the AFP in malignant liver diseases whether primary or secondary to a known primary lesion. Second, is to conduct a comparative study between AFP elevation and serum alkaline phospatase in an attempt to evaluate the function of the liver and the severity of its affection by the tumour. Third, is to assess the role of AFP estimation in the diagnosis of malignant liver lesions, in a trail to improve the accuracy of clinical diagnosis and aid in early detection of liver neoplasms.

# Review of Literature

## ALPHA-FETOPROTEIN

## [AFP]

Alpha fetoprotein (AFP) is an alpha-1-globulin present in high concentration in fetal serum particularly in early gestation (Alpert et al., 1971), but in very small amounts in the serum of normal adults (Ruoslahti and Seppala, 1971).

It was first detected in human fetal serum by Bergstrand and Czar in 1956 during electrophoresis experiments of plasma proteins of fetus of a few weeks old, as a protein which migrated between albumin and alpha-1-globulin.

The production and secretion of AFP into the fetal serum begins with the differentiation of the fetal liver. Early in gestation, the yolk sac and the mucosa of the gastrointestinal tract synthesize AFP, but by the eighth week, the liver surpasses the yolk sac in size and becomes the major site for AFP synthesis (Gitlin et al., 1972).

AFP synthesis increases rapidly and by 13 weeks of gestation reaches a peak level of 3-4mg/ml (Gitlin and Boesman, 1966). From the 14th to the 32nd week, the AFP concentration declines to a level of 2mg/ml (Pandey et al., 1986). At the age of two

years, AFP level reaches 1 to 2ng/ml or 1: 20,000 of that present at birth, and this low level persists into adult life (Nolon, 1979).

Abelev in 1968, tried to explain the dynamics of AFP synthesis during fetal life as following: AFP synthesis is felt to be the property of specialised cells of liver parenchyma. With the maturation of the fetus, an alternative type of hepatocyte is produced and becomes the predominant liver cell. This type of cells produces the adult serum proteins but cannot make AFP. Meanwhile Pandey et al in 1986 have explained the decrease in the fetal AFP serum level as a disproportionately progressive increase in fetal growth and its expanding body fluid volume, rather than a decrease in hepatic AFP synthesis.

One of the main routes by which alpha-fetoprotein is eleminated from the fetal circulation is through excretion with fetal urine into amniotic fluid (Weiss et al., 1976).

During pregnancy, the maternal serum AFP increases with advancing gestation due to fetal placental transfer (Seppala and Ruoslahti, 1972b). Normally this increase is transient and falls quickly (Seppala and Ruoslahti, 1972a).

Properties of alpha-fetoprotein:

AFP is a glycoprotein with a molecular weight of approximately 70,000Mw; slightly larger than human serum albumin (Alpert et al., 1972).

The molecule appears to be composed of a single polypeptide chain containing about 30 amino acids. These amino acids of alpha fetoprotein isolated from fetal sera, did not show any considerable difference when compared with AFP isolated from sera of patients with hepatomas (Ruoslahti et al., 1971). Carbohydrates in the form of hexose, sialic acid, and hexosamine are present to the extent of about 3% of the molecule (Alpert et al., 1972).

Fetal and cancer AFP also have similar physical and electrophoretic properties and give a reaction of immunological identity (Ruoslahti et al., 1971).

AFP is not identifiable by the routine electrophoretic techniques. The detection of alpha-fetoprotein depends on its unique antigenic determinants and the development of types of specific immunoassays, by the use of specific antisera. Antisera can be elicited by immunization of a number of heterologous species [e.g rabbit, guinea pig, sheep, goat and horse) with

purified alpha-fetoprotein fetal or hepatoma sera. Antisera to alpha-fetoprotein does not react with any other known human serum protein (Ruoslahti and Seppala, 1971).

**Methods of Assay:**

Since the description of AFP, there have been numerous attempts to improve the sensitivity and specificity of the assay method used to detect AFP.

The early experimental and clinical observations were made with a relatively crude agar gel-diffusion (AGD) technique that had a limited sensitivity of probably no more than 1 to 10ng/ml. At this level of sensitivity, the detection of alpha-fetoprotein became widely used as a diagnostic test for hepatocellular carcinoma, with few false positive reactions (Purves et al., 1970).

A number of other more sensitive techniques had been used in more recent studies; like counter immunoelectrophoresis (CIEP), electroimmunodiffusion (EID) and latex fixation. These methods can detect approximately 250 to 500ng/ml of AFP. Haem-agglutination, double antibody radio-labeled electroimmuno diffusion (REID) and immunoenzymatic assays can detect as little as 50ng/ml. Modifications of immunoenzymatic and (REID) have been reported to be able to detect 1 to 10ng/ml.

Radioimmunoassay (RIA) measuring the competitive inhibition by human sera of radiolabeled purified AFP, is able to detect a few nanograms per milliliter of fetoprotein (Alpert, 1976).

More recently, enzyme-linked immunosorbant assay (ELISA) is used with some advantages over radioimmuno assay as it requires only minimal equipment, it is less time consuming, less expensive and much more easy to perform (Belanger et al., 1973).

**Normal Levels:**

The concentration of AFP in the human serum reaches a maximum of 3mg/ml at about 13 weeks of gestation (Gitlin and Boesman, 1966). Subsequently its concentration decreases until at birth it is approximately 1% of the peak level. At the age of two years, AFP level reaches 1 to 2ng/ml (Nolon, 1979).

In adult serum, AFP levels are generally below 10ng/ml (approximately 8 IU) by radioimmunoassay method depending upon the fact that one international unit equals from 0.54 - 1.81ng of AFP, a more recent study indicates that one IU equals 1.21 $\pm$ 0.81ng of AFP (Trichopoulos et al., 1980).

Physiological function of AFP:

Since the time of discovery of alpha-fetoprotein, its function in human fetus has remained largely unknown. But due to its early appearance in life, rapid synthetic rate and similarity to albumin in molecular weight and phsico-chemical characters, it has been suggested that AFP may be a substitute for albumin and that albumin replaces AFP in the same way the adult haemoglobin replaces fetal haemoglobin (Abelev, 1971). On the same principle, Alpert and his coworkers (1972) suggested that AFP must function as an osmotically active molecule protecting the intravascular volume.

It was postulated by Smith in 1972 that alpha-fetoprotein helps to maintain the fetus as an allograft to the mother in a genetically incompatible environment. Also in 1984, Markatz and his coworkers have found that alpha-fetoprotein has an immunoregulatory function during pregnancy and this function prevents the rejection of the fetus by the mother.

# AFP And Its Relevance To Human Diseases

## 1. AFP in liver Diseases

With the use of sensitive methods of assay, raised levels of AFP have been detected in the sera of patients with various forms of liver diseases other than liver tumors (Bloomer et al., 1973). The serum concentrations frequently increased in those disorders in which there is prominent hepatocellular necrosis, inflammation and regenerative response (Alpert and Feller, 1978).

Repeated determinations over a period of time will, therefore, increase the diagnostic reliability of levels of AFP: normal or flactuating values suggesting a benign process, while rising levels (unless the tumor is removed) being characteristic of malignancy (Ruoslahti et al., 1974).

### (1) AFP and liver cirrhosis:

Smaller increases in AFP concentration may occur in patients with a variety of liver diseases such as cirrhosis (Kew, 1974) and evidence from experimental animals suggests that this rise could be used as a marker for the regenerative response of the liver after massive liver damage (Sell et al., 1974).