Identification of High-Risk Pregnancies by Maternal Serum Alfa Fetoprotein

T. Eswar Ganesh Babu*, S.A. Mastan, M. Sai Sowjanya, G. Rajesh, K. Harshitha, A. Madhavi, U. Haritha, B. Sindhulekha, P. Sivanrendra Babu

Department of Pharmacy, Malineni Perumallu Educational Society and Group of Institutions, Vatticehrukuru [M] Guntur {Dist} 522017 A.P, India

Abstract
Alfa- Feto- protein (AFP) is a protein produced by the baby in the womb. The protein is normally found in the blood stream of the mother, which tends to be elevated or lowered in the blood of the women carrying babies with certain abnormalities it is an Albumin like glycoprotein with a molecular weight of 70,000 D. Is a formed in the yolk sac. Non differential liver cells and the fetal gastro- intestinal tract. Elevated MSAFP levels have also been associated with Chromosome abnormalities, Omphalocele and gastroschisis and rarely congenital, additionally elevated MS AFP levels have associated with an increased risk for adverse perinatal out comes including preeclampsia, premature labor, low birth weight, placental abruption and stillbirth.

Keywords: Maternal serum; alfa fetoprotein; Amniotic fluid; Open neural tubular defects

Introduction
- A protein detected in fetal serum as an extra band in the Alfa-1 region. Since it was not seen in normal adults it was called AFP.
- Synthesized in yolk sac and fetal liver.
- Peak fetal serum concentration is 300mg reached during the end of the first trimester (Hook, 1981).
- The fetal liver produces AFP until 30 weeks of gestation and then stops rather abruptly.
- A specific function of AFP has not been found. Fetus with a genetic deficiency for production of AFP have been reported without adverse effects.
- Genes for both proteins are located in chromosome 4.
Both have similar molecular weight (69,000 Daltons).
Both have the same amount of negative charges.
Circulating time for AFP is 4 days.

Anti-sera for AFP do not cross react with albumin therefore it can be used to detect it in maternal serum. Maternal serum Alfa fetoprotein testing is done to screen for open neural tube defects (ONTDS). Anencephaly has an incidence of 1 in 2000 and Spina bifida 1 in 1000. Making NTE S one of the more common birth defects. Elevated levels of MSAFP are observed in 80-90% of affected pregnancies (Fuder et al 1996).

Elevated MSAFP levels have also been associated with Chromosome abnormalities, Omphalocele and gastroschisis and rarely congenital nephrosis of the kidney. Additionally, elevated MSAFP levels have been associated with an increased risk for adverse perinatal out comes including preeclampsia and premature labor, low birth weight, placental abruption and stillbirth. (McKusick, 1992)

The association between trisomy 18 and lower levels of all three analyses is also well established. (Fuder et al 1996.)

The optimal time for maternal serum screening is between 15- and 18-weeks’ gestation. If the test results are normal, no further follow-up is required. If the test results are suggestive of an open neural tube defect or chromosome abnormality, and the gestation date is verified, further testing is recommended. (Fuder et al 1996.)

Women with positive test results are offered the option of having an ultrasound examination to confirm the gestation age of the fetus and to identify and obvious structural defects or other factors, such as fetal demise or twins, that might explain the altered level of maternal serum markers.

Should the cause of the abnormal MSAFP remain unresolved, women are given the option of having an amniocentesis for chromosome analysis and amniotic fluid AFP determination (Briggs et al 1994)

If an NTD is suspected, the level of acetylcholinesterase will be determined. Acetylcholinesterase is a component of the fetal cerebrospinal fluid and, if found in the amniotic fluid; confirms the diagnosis of an open neural tube defect (Baker and Rudolph, 1971)

MSAFP screening test result shows (Bendon,1991; Bennet and Harrold, 1976)

AFP levels within normal range indicate low risk for fetal anomalies.
Higher AFP level indicates an increased risk of:
• Neural tube defects in the fetus such as Anencephaly or Spina bifida.
• Other fetal abnormalities.
• Threatened abortion.

Lower AFP levels indicate an increased risk of:
• Trisomy-21 fetus (Down syndrome).
• Other rare genetic disorders.

Other reasons for abnormal reports on maternal blood AFP levels (Boue and Boue, 1978; Buchsbaum and Caruso, 1969)

• AFP levels change with the gestation period. If the gestation is not calculated accurately, the AFP results may not be corresponding and thus may show up as abnormal.
• In multiple pregnancy (like twin pregnancy) the AFP levels are high.
• If the pregnant mother has insulin dependent diabetes AFP levels are 10% lower than the normal average AFP level.
• Maternal weight: obese pregnant women have low AFP levels due to dilution of AFP in larger blood volumes.

Methods and Materials

Patients and Blood Samples
The blood samples were collected from 20 patients, 16th week of gestation during pregnancy. The patients were confirmed by a gynecologist. The subjects were female ranging 18-38 years.

Estimation of MSAFP
To estimate the amount of MSAFP in serum using chemiluminescence method threw “ELECSYS SYSTEM 1010”, Enzyme Linked Electro Chemiluminescence immune assay systems, Bablok et al., (1988) was adopted.

Specimen Collection and Preparation
Blood was obtained by venous arm puncture in a heparinised tube plasma separated by centrifuge at 3000 rpm for 15 minutes.

Plasma treated with sodium heparin, EDTA-K3 or sodium citrate. When sodium citrate is used, the results must be corrected by +10%.

heat- inactivated samples are not used. Sample and control are not stabilized with azide. The patient’s sample I made calibrated and controlled at ambient temperature (20-25°C) before measurement. Because of possible evaporation effects, samples, calibrators and controls on the analyzer should be measured within two hours.

Reagents
Elecsys AFP reagent kit.
M Streptavidin-coated microparticles:
Streptavidin-coated microparticles, 0.72 mg/ml, binding capacity: 470 ng biotin/mg microparticles; preservative.

R1 Anti-AFP Ab ~ biotin:
Biotin labeled monoclonal anti-AFP antibodies (mouse) 4.5 mg/dl; phosphate buffer 100 mmol/l, pH 6.0; preservative.

R2 Anti-AFP ab ~ Ru(bipy):
Monoclonal anti-AFP antibodies (mouse) labeled with ruthenium complex 12.0 mg/l; phosphate buffer 100 mmol/l, pH 6.0; preservative.

Procedure
Sandwich principle, total duration of assay: 18 minutes.

1st incubation: 10 µl of sample, a biotinylated monoclonal AFP-specific antibody and a monoclonal AFP-specific antibody labeled with a ruthenium complex react to form a sandwich complex.

2nd incubation: after the addition of streptavidin-coated microparticles, the complex becomes bound to the solid phase via interaction of biotin and streptavidin.

The reaction mixture was aspired into the measuring cell where the micro particles are magnetically captured onto the surface of the electrode. Unbound substances are then removed with ProCell. Application of a voltage to the electrode then induces chemiluminescent emission, which is measured by photomultiplier.

Results are determined via a calibration curve that is an instrument-specifically, generated by 2-point calibration and a master curve provided via the reagent bar code.

Values are expressed as ng by elecsys 1010 (automatic analyser). (Baker and Rudolph, 1971; McKusick 1992; Ferguson-Smith and Yates, 1984).

Alpha-fetoprotein (AFP) Blood Test
The most widely used biochemical blood test for liver cancer - hepatocellular carcinoma (HCC) is Alpha-fetoprotein (AFP), which is a protein normally made by the immature liver cells in the fetus. At birth, infants have relatively high levels of AFP, which fall to normal adult levels by the first year of life. Also, pregnant women carrying babies with neural tube defects may have high levels of AFP. (A neural tube defect is an abnormal fetal brain or spinal cord that is caused by folic acid deficiency during pregnancy.)

In adults, high blood levels (over 500 nanograms/milliliter) of AFP are seen in only three situations:
- HCC
- Germ cell tumors (cancer of the testes and ovaries)
- Metastatic cancer in the liver (originating in other organs)

Several assays (tests) for measuring AFP are available. Generally, normal levels of AFP are below 10 ng/ml. Moderate levels of AFP (even almost up to 500 ng/ml) can be seen in patients with chronic hepatitis. Moreover, many patients with various types of acute and chronic liver diseases without documentable HCC can have mild or even moderate elevations of AFP.

The sensitivity of AFP for HCC is about 60%. In other words, an elevated AFP blood test is seen in about 60% of HCC patients. That leaves 40% of patients with HCC who have normal AFP levels. Therefore, a normal AFP does not exclude HCC. Also, as noted above, an abnormal AFP does not mean that a patient has HCC. It is important to note, however, that patients with cirrhosis and an abnormal AFP, despite having no documentable HCC, still are at very high risk of developing HCC. Thus, any patient with cirrhosis and an elevated AFP, particularly with steadily rising blood levels, will either most likely develop HCC or actually already have an undiscovered HCC.

An AFP greater than 500 ng/ml is very suggestive of HCC. In fact, the blood level of AFP loosely relates to (correlates with) the size of the HCC. Finally, in patients with HCC and abnormal AFP levels, the AFP may be used as a marker of response to treatment. For example, an elevated AFP is expected to fall to normal in a patient whose HCC is successfully removed surgically (resected).

There are a number of other HCC tumor markers that currently are research tools and not generally available. These include des-gamma-carboxyprothrombin (DCP), a variant of the gamma-glutamyltransferase enzymes, and variants of other enzymes (e.g., alpha-L-fucosidase), which are produced by normal liver cells. (Enzymes are proteins that speed up biochemical reactions.) Potentially, these blood tests, used in conjunction with AFP, could be very helpful in diagnosing more cases of HCC than with AFP alone.

Results & Discussion
Biochemical Estimation
In the present study, we analysed the levels of AFP in 9 abnormal pregnancy subjects compared with age and sex matched 11 normal pregnancy subjects. Table 1 shows subjects and parameters of the study.

| Table 1: Subjects and parameters of the study |
| Human Subjects | Parameters |
| Serum | Alfa fetoprotein |
Table- 2: Case histories of the subjects investigated of abnormal subjects

| S.N. | Subjects       | Age (16th week of gestation during pregnancy) | Sex     | Total Number of patients | Clinical status                        |
|------|---------------|---------------------------------------------|---------|--------------------------|----------------------------------------|
| 1    | Normal        | 18-30                                       | Female  | 11                       |                                        |
| 2    | Above normal  | 27-36                                       | Female  | 6                        | ONTD.Ex: anencephaly & Spina bifida     |
| 3    | Below normal  | 29-42                                       | Female  | 3                        | Down syndrome (Trisomy-21)             |

Table 3: Levels of AFP in Serum of Normal and Fetal Abnormality Patients

| Parameter | Normal subjects | Abnormal subjects |
|-----------|-----------------|-------------------|
|           |                 | Above normal      | Below normal     |
|           |                 | (1) 56.51 ng/ml   | (1) 3.65 ng/ml   |
|           | (2) 45.14 ng/ml | (2) 72.13 ng/ml   | (2) 9.37 ng/ml   |
|           | (3) 24.34 ng/ml | (3) 127.40 ng/ml  | (3) 2.13 ng/ml   |
|           | (4) 35.77 ng/ml | (4) 165.40 ng/ml  |                 |
|           | (5) 30.25 ng/ml | (5) 149.90 ng/ml  |                 |
|           | (6) 23.37 ng/ml | (6) More than 100 ng/ml | |
|           | (7) 26.07 ng/ml |                   |                 |
|           | (8) 22.50 ng/ml |                   |                 |
|           | (9) 35.80 ng/ml |                   |                 |
|           | (10) 24.60 ng/ml|                   |                 |
|           | (11) 29.70 ng/ml|                   |                 |

Case histories of AFP

The Table 2 shows the case Histories of the subjects investigated of abnormal subjects, i.e., 6 above normal and 3 below normal subjects and 11 normal pregnancy subjects were selected for the study. All the normal subjects in the age group of 18-30 years. The abnormal subjects in the age group of 27-36 years for above normal and 29-42 for below normal.

Levels of AFP in Serum of Normal and Fetal Abnormality Patients

The Table 3 shows the serum AFP level in normal and abnormal pregnancy women. The normal level of serum AFP is in the range of 11.92-60.9 ng/ml. From the table it is observed that AFP significantly increased in abnormal pregnancy (above normal) and AFP significantly decreased in abnormal pregnancy (below normal) when compared to normal pregnancy subjects.

The present study highlights the relation between pregnancy risk in abnormal pregnancy women by investigating alfa fetoprotein (Bendon, 1991)

The results were compared with equal number of normal pregnancy women with the age group of 18-38 years.

The levels of alfa fetoprotein were significantly increased in above normal subjects, while significantly decreased in below normal subjects when compared to the normal pregnancy control subjects.

We observed markedly elevated levels of alfa feto protein in pregnancy women. High maternal serum alfa feto protein is an indicator of risk of neural tube defects in the fetus such as Anencephaly and Spina bifida. In this condition, the chromosome will be altered at 18 set of chromosomes (Trisomy 18). (Bennet and Harrold, 1976)

Elevated MSAFP other than NTD are under estimation of gestation age, in as much as MSAFP increases as gestational progresses; multiple gestation with 60% of twins and almost and triplets showing and elevated value, threatened abortion, presumable due to fetal blood escaping into the maternal circulation, Rh diseases and other conditions associated with fetal edema; anomalies other than NTD; and fetal demise.

In the present study, we observed the levels of AFP were significantly decreased. The decreased level of MSAFP is found in Down syndrome (Mongolism). This is the most common chromosomal defect.

A normal healthy cell has 46 chromosomes while a cell with DS has 47 chromosomes. The extra chromosome is with the 21st set, and so-called trisomy 21.

The abnormal genes lead to congenital malformation, the faces of the infants are mongoloid, with narrow slanting
closely set palpebral fissures. The tongue is thick and fissured and the palatal arch often high. Fingers are stubby and the hands present clear cut dermatoglyphic patterns, particularly a similar line mental retardation. (Hook, 1981)

The risk of birth of live born infants with Down syndrome is due to age. The risk increase to about 1 in 100 by age 40 and 1 in 32 by age 45 (Corry and Whyler, 1984).

Summary and Conclusion

In the present study investigated maternal serum in pregnant women. Alfa fetoprotein is one of the many proteins found in the blood system of pregnant women. Every fetus produce AFP, it is similar to albumin located in chromosome no.4 and molecular weight 69,000 Dalton.

A total of 20 pregnancy women in the age group 18-30 were selected for the study. Out of 20 pregnancy women 11 is normal in age group 18-38. 6 is above normal in the age group of 21 - 30 and 3 is below normal in the age group of 20 - 25. Blood samples were collected and analyzed for the serum Alfa feto protein. The results were compared with normal pregnancy women.

The levels of serum alfa fetoprotein were significantly increased in above normal pregnancy as compared to normal subjects. This could be due to neural tube defects including anencephaly and spinabifida (Trisomy 18). In this condition the chromosomes will be altered.

The levels of serum Alfa feto protein were significantly decreased in below normal pregnancy as compared to normal subjects. This could be due to Down syndrome (Trisomy 21). This is most common chromosomal defect.

This study concludes that every pregnant woman must do the MSAFP screening test between 15-20 weeks from the first day in the last menstrual period. If find any alteration better to go further investigation and treatment, such as Chromosomal analysis of the amniotic fluid after Amnocentesis, sophisticated ultrasound exam and repeat maternal AFP test. If find more complications can terminate the pregnancy.

Authors’ Contribution

All authors equally contributed at every stages of research work, Manuscript preparation and final approval of the manuscript.

Conflict of Interest

The authors declare that there is no conflict of interest with present publication.

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