

# STUDY OF LEVELS OF ALFAFETO PROTEIN (A.F.P.) LEVELS IN AMNIOTIC FLUID IN NORMAL PREGNANCIES AND ITS CO-RELATION WITH GESTATIONAL AGE

by

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Efforts have been made repeatedly to find a suitable method for diagnosis of early pregnancy and estimation of gestational age. In clinical practice, one of the most fascinating areas is the use of A.F.P. assay in antenatal diagnosis. Gitlin and Boesman (1966) were the first to detect this protein in amniotic fluid from 6 weeks of gestation onwards.

Seppala (1972) observed a decrease in the level of this protein with advancing pregnancy. The present study was undertaken to establish and study the correlation between amniotic fluid A.F.P. and gestational age.

## Material and Methods

One hundred and seventy-four cases attending M.L.B. Medical College Jhansi's outpatients department and indoor wards of Obstetrics and Gynaecology were taken for this study. Amniotic fluid samples were taken from patients coming for

M.T.P., antenatal cases presenting in various trimesters, during labour or at the time of caesarean section. A detailed history was taken trimesterwise and precise date of L.M.P. was documented to know the exact period of gestation.

## Collection of Amniotic Fluid

Samples were taken in first trimester M.T.P. patients by introducing vaginally a long needle after dilatation of cervix by Hegar's dilators under aseptic precautions and abdominally in 2nd and 3rd trimesters by transabdominal paracentesis. During labour samples were taken by introducing a needle in the bulging membranes to avoid contamination with vaginal discharge, urine or blood. At the time of caesarean section samples were taken by introducing a needle before incising the uterus.

After collection, the samples were immediately centrifuged at 3,000 r.p.m. for 5 minutes and supernatant fluid was kept in deep freeze at 0°C and taken out only at the time of use.

Qualitative screening of samples was done by immune precipitation technique as described by Pasendorfer *et al* (1970) and samples showing precipitation bands under oblique illumination were subject-

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ed to quantitative determination of A.F.P. using dimensional radial immunodiffusion method of Mansini *et al* (1965). Different concentrations of standard A.F.P. antigen (Behring Werke A.G. Marburg, West Germany) were used as known positive control for the test.

#### Evaluation of Results

The distance of rocket in mm was plotted against the known concentration of standard A.F.P. antigen on log scale. The distance of the rockets of unknown samples were noted and read on this graph, so as to find out the concentration of A.F.P. antigen (mgm/ml.) in these samples.

#### Observations and Results

Normal antenatal cases included in this study numbered 102 and cases admitted for M.T.P. 72. The distribution of these cases trimesterwise is shown in Table I.

The values of A.F.P. obtained in cases of M.T.P. and in antenatal and intranatal cases is shown in Table II and Table III.

As seen in the above Tables, the A.F.P. level (mean) upto 12 weeks in the amniotic fluid was 1700 mg/ml, between 13-16 weeks between 2700 mg/ml and 2750 mg/ml., between 17-20 weeks 1000 mgm/ml. and between 21-27 was 500 mgm/ml. In the first group the values obtained in cases of M.T.P. and antenatal cases were approximately the same. In the antenatal cases A.F.P. levels in 3rd trimester was very low. It was 500 mg/ml. only in 1 case and was not detected in the rest of the cases. During labour A.F.P. was not detected in any case.

#### Discussion

From the preceding observations it becomes evident that peak mean A.F.P. in amniotic fluid in mgm/ml is 2700 between

TABLE I  
Distribution of Cases Trimesterwise

Sl. No.	Period of gestation	Clinical presentation	No. of cases	Total
1.	1st trimester (upto 12 weeks)	—For M.T.P.	46	72
2.	2nd trimester (Between 13-28 weeks)	—For M.T.P.	26	
3.	3rd trimester (Between 29-39 weeks)	—A N cases	50	102
4.	At term with labour pains	—A N cases	35	
		In labour pains for delivery.	17	

TABLE II  
Values of A.F.P. in Cases Admitted for M.T.P.

Sl. No.	Period of gestation	No. of cases	Range of A.F.P. (mgm/ml.)	Mean A.F.P. (mg./ml.)
1.	Upto 12 weeks	46	1500-1900	1700
2.	Between 13-16 weeks	12	2500-2900	2700
3.	Between 17-20 weeks	14	800-1200	1000

TABLE III  
Values of A.F.P. in Ante and Intra Natal Cases

Group of cases	Period of gestation	No. of cases	Range of AFP (mgm/ml.)	Mean AFP (mgm/ml.)
Group I	2nd Trimester			
	—Between 13-16 weeks	22	2500-3000	2750
	—Between 17-20 weeks	14	800-1200	1000
	—Between 21-27 weeks	14	800- 700	500
Group II	3rd Trimester			
	—28 weeks and above	35	In 1 case 500 mg/ml., not detected in the rest.	In 1 case 500 mg/ml., not detected in the rest of the cases.
Group III	During labour	17	Not detected.	

13-16 weeks of pregnancy. Levels upto 12 weeks are lower than this and are 1700 mgm/ml. The levels show a decline after 16 weeks and is 1000 mg. between 17-20 weeks, 500 mg. between 21 and 27 weeks. After 28 weeks, the levels become still lower and are not detected by the method used—only 1 case showed a level of 500 mgm/ml. The same pattern of decline continues and during labour and A.F.P. is not detected.

The cause of A.F.P. not being detected in our studies is the levels of A.F.P. which were below the lower limit of sensitivity of the method used. Kelkar and Nagar (1976) detected A.F.P. concentration by radioimmuno diffusion method and observed similar results. The method used to detect A.F.P. levels in A.F. has the inherent limitation of being sensitive only above 500 mg/ml levels.

Our findings are comparable to those of Gitlin Boesman (1966) who also found a peak value of A.F.P. between 13-15 weeks and then a gradual fall till term.

Our findings are also in accordance with that of Seppala (1972) who determined A.F.P. level in 2nd trimester of pregnancy

between 2600 mg/ml. and 2300 mg/ml. and during 3rd trimester between 5-535 mg/ml.

Kjessler *et al* (1977) found maximum level of A.F.P. between 12-14 weeks of gestation (25-45 mg/L) which decreases to 3-25 mg/L in 2nd trimester and further decreases to 0.5 mg/L at term.

#### Conclusion

A study of A.F.P. in amniotic fluid was done in all trimesters of pregnancy and a definite correlation between gestational age and the levels of A.F.P. was found to exist. Maximum level was between 13-16 weeks of gestation which decreased gradually and after 28 weeks it was not detected by the method used, because of levels of A.F.P. below the lower limit of sensitivity.

However, the importance of a single observation is not as much significant as repeated estimations showing a progressive rise upto 14-16 weeks and then a decline.

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