STUDY OF BIOCHEMICAL PARAMETERS IN AMNIOTIC FLUID FOR ASSESSMENT OF FOETAL MATURITY IN CASES OF NORMAL PREGNANCY

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ABSTRACT: Assessment of foetal maturity had been proven of value in evaluating the foetal condition. Accurate assessment of foetal maturity is essential for the proper timing of delivery in various risk pregnancies. Amniotic Fluid analysis for foetal maturity had been of proven value. In the present study, study of biochemical parameters in amniotic fluid in respect of Creatinine, Uric Acid, Urea, Total Proteins, and Electrolytes i.e. Sodium, Potassium and Chloride has been done, along with Serum Electrolytes. Standard methodologies were adopted. The observations in the present study correlated with the works of Chadick et al and Pitkin and Zwirek. The levels of Creatinine, Uric Acid and Urea in Amniotic Fluid showed elevation, while Total Proteins and Serum Sodium showed a decline, as gestation progressed. The Serum and Amniotic Fluid Potassium and Chloride levels remain almost constant throughout the pregnancy. Thus, it is observed that the use of multiple parameters is desirable for accurate assessment of foetal maturity.

KEYWORDS: A. F.: Amniotic Fluid, Cr.: Creatinine, U.A.: Uric Acid, wks: weeks, MTP: Medical Termination of Pregnancy, S.D.: Standard Deviation.

HOW TO CITE THIS ARTICLE: K. V. Leela, Kasi Babu. "Study of Biochemical Parameters in Amniotic Fluid for Assessment of Foetal Maturity in Cases of Normal Pregnancy". Journal of Evidence based Medicine and Healthcare; Volume 2, Issue 48, November 16, 2015; Page: 8394-8399, DOI: 10.18410/jebmh/2015/1143

INTRODUCTION: Accurate estimation of Fetal maturity is essential for the proper timing of delivery in various high risk pregnancies i.e. clinical obstetrical conditions before fetal demise. The recognition of the clinical usefulness of Amniotic Fluid (A.F.) obtained by Amniocentesis under Ultrasound guidance for assessment of the fetus as recognized in 1956, when Bevis[1] demonstrated increased levels of Bilirubin in the A.F. of infants with Erythroblastosis foetalis. So many methods of assessing the Foetal maternity like Cytological, Sonar, Biochemical, (Bilirubin, Creatinine, L / S ratio, Cytology, Electrolytes, Proteins, Urea, Enzymes, Hormones). Hence, an endeavor is made to study the Bio-chemical profile of the A.F. at various periods of gestation and evaluate the possible relationship between these constituents and Foetal maturity.

The Bio-chemical assessment methods have been proven of value in evaluating the fetal condition. With the recent development and refinement of Bio-Physical techniques of Fetal surveillance (Johnson et. al.)[2].There has been a progressive decrease in the clinical use of Bio-chemical markers as indicators of fetal status. These Bio-Physical methods include measurements such as of A.F. volume, fetal tone, Fetal breathing, movements, Non-stress test, in placental grading. Thus at best, bio-chemical fetal assessment may be used in limited clinical circumstances as adjunct to rather than definitive factor in the clinical management of High Risk Pregnancies.

Submission 25-10-2015, Peer Review 26-10-2015,
Acceptance 31-10-2015, Published 14-11-2015.
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DOI: 10.18410/jebmh/2015/1143

The A.F. averages to 50 ml at 12 wks gestation, 400 ml at mid pregnancy, maximum of 1 litre, at 36-38 wks. Later it decreases as the term approaches if pregnancy is prolonged, the A.F. becomes relatively scanty (Elliot and Inman)[3]. The comparison of A.F. in early pregnancy is as that of a dialysate of maternal serum. Thus A.F. is initially Isotonic with maternal plasma. As fetal skin is permeable to water in early pregnancy, then the fluid could be a transudate of fetal plasma. As the pregnancy advances, the A.F. becomes progressively hypotonic, everything chiefly to a decline in sodium concentration, and decrease in osmolar concentration, and an increase in Creatinine, Urea, and Uric Acid (U.A.)(T.A. Doran, et al)[4]. The concentration of chloride does not fall with that of sodium possibly because small amounts of HCl reach the A.F. from the fetal stomach (Hutchinson, et al)[5]. Simple diffusion across the fetal mucus membranes in both directions is continuous in late pregnancy, so that abnormal Bio-chemical changes in maternal blood may be reflected in A.F.(Bor NM., Corbit JD., Chasteney EA., et al)[6].

The importance of Creatinine in predicting foetal maturity was first emphasised by Pitkin & Zwirek 1967[7], and Droegemuller W., Jackson C., Makowski EL., and Battaglia, FC. studies[8] and Parmley & Muller[9]. A positive correlation between Creatinine content and foetal distress was suggested by Dr.Roychowdhury et al[10] and Kusumlatha, et al 1984[11]. Electrolytes concentration in A.F. at various periods of gestation was measured by Gullibrand G., Lind F., Benzie RJ[12] in A.F. collected early in pregnancy and found that Alannine, Glutamic Acid, Lysine, Protein, threonine, glycine, valine were the most common Amino acids. These Amniotic acids comprise 75% of the total and were found by them being the same as these are found in highest concentration in plasma.

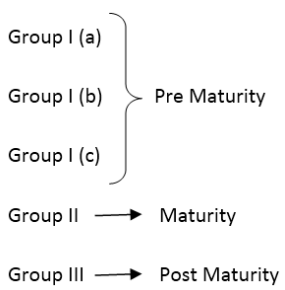
AIM OF STUDY: The present study comprises the assessment and evaluation of foetal maturity by study of

clinical and Bio-chemical parameters in A.F. in respect of Creatinine, Uric Acid, Urea, Total Proteins, Sodium, Potassium, Chloride, and Serum electrolytes like Sodium, Potassium, Chloride in 17-23, 24-27, 30-35, 36-40 and 41 and above weeks of normal pregnancy to evaluate the possible relationship between these constituents and foetal maturity.

The aim of monitoring foetal condition is to ensure the functional integrity of cells, because of the tolerance of different tissues to the changes in internal environment. The state of the most vulnerable system should be assessed when a disturbance in homeostasis is suspected. Foetal maturity essentially relates to the physiologic development of the various foetal tissues and organ systems as they mature at different rates and is related to fetal aging and growth. Assessing the foetal state without jeopardising to the fetus itself has prompted investigation of the diagnostic potential of A.F. rather than fetal mood sampling, Thus, the investigation results of A.F. analysis gained more importance as a means of diagnostic parameters for foetal maturity & foetal wellbeing. All the A.F. indices of foetal maturity are better predictions of maturity than of immaturity.

MATERIALS AND METHODS:

Source: The present studyselected 50 cases of normal pregnancy without any complications. Samples of A.F. were obtained from cases of normal pregnancy, admitted in the Obstetrics and Gynecology wards, outpatient labour room of King George Hospital, Visakhapatnam, Andhra Pradesh. The samples were collected from women irrespective of age and parity. The women subjected to study were divided into the following groups:



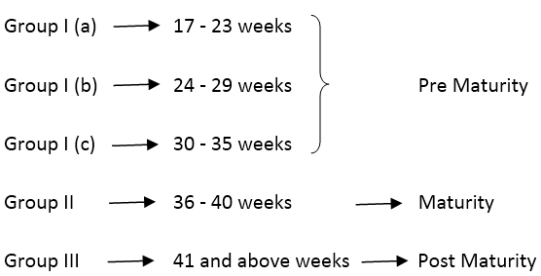
Sample Collection: The sample collection of A.F. was performed under strict aseptic precautions by the Obstetricians and that sample was taken for analysis. In the first half of normal pregnancy, A.F. samples were collected from cases of MTP and Amniocentesis had been done by the Obstetrician from abdomen or vaginally depending upon the choice to be employed. A.F. samples in the second half of normal pregnancy were collected from antenatal patients as well as patients in early labour with intact membranes. In these antenatal patients who are not in labour, amniocentesis had been done by concerned labour room obstetricians by trans-abdominal route and these samples were collected with great care to avoid risk to fetus. In early labour cases, obstetricians tapped the fore- waters and the Amniotic fluid samples were obtained.

All the collected samples of 15-17 ml volume of A.F. had been protected from exposure to light during their transfer from patients side to investigative work bench that takes a matter of only a few minutes. The samples are centrifuged at 3 000 rpm for 10 minutes to eliminate the epithelial cells, red cells and other particulate matter and to obtain a clear sample. If the collected samples were much mucosium stained and much blood stained, such samples were discarded. When the samples are turbid, centrifugation at 12 000 rpm using a high speed centrifuge at 0° C for 30 minutes was done. Soon after centrifugation, samples are analysed for Bio-chemical constituents. But, in some cases when there is a delay, the centrifuged samples are refrigerated for a period of 3-4 hours, then they were analysed.

METHODS: After thorough clinical examination was done by the obstetrician, following were the investigations done.

1. Estimation of Creatinine(JAFFE's method, 1945)[13]
2. Estimation of Uric acid(URICASE - POD method)[13]
3. Estimation of Urea(DAM method)[13]
4. Estimation of Total Proteins(Henry method)[13]
5. Estimation of Electrolytes - Sodium, Potassium, Chlorides(Flame photometry)[13]

OBSERVATIONS: The study consists of 50 cases of normal pregnancy without any complications. The samples were collected from women irrespective of age and parity. The women subjected to study were divided into the following groups.



The values obtained are represented to the corresponding gestational age groups in the tables (Table 1, Table 2(a), Table 2(b), Table 2(c), Table 2(d), Table 2(e), Table 3).

The Creatinine values show an increasing trend as the gestation advances. There is much level of significance among the groups (Table 2(a) (fig 1).

The Uric Acid values show an increasing trend as the gestation advances. There is much level of significance among the groups (Table 2(b) (fig 1(a)).

The Urea values show an increasing trend as the gestation advances. There is much level of significance among the groups (Table 2(c) (fig 1(b)).

The Total Protein levels of a declining trend as the gestation advances. There is much level of significance among the groups (Table 2(d) (fig 1(c)).

The Sodium values in A.F. show a declining trend as the gestational age advances. In Serum, the Sodium values are almost remain constant at 134 meq per litre (Table 2(e)).

The Potassium and Chloride values in A.F. and Serum, are constant throughout pregnancy (Table 2(e)).

The Creatinine, Uric Acid and Urea have a significant positive correlation whereas Total Protein and A.F. Sodium showed a significant negative correlation (Table 3).

DISCUSSION: The commonest cause predominantly responsible for neonatal deaths is prematurity. Various techniques have been tried to assess the Fetal age in such high risk pregnancies. In such cases a test which has high percentage of accuracy will be of much importance. In the present study Creatinine, Uric Acid, Urea, Total Protein levels had been estimated in FIVE gestational age groups. The A.F. and Serum levels of Sodium, Potassium and Chloride are also estimated with a view to delineate the correlation between the values of these constituents as the gestational age advances.

The A.F. Creatinine concentration level was noted with increasing gestational age in the present study. Other workers have also reported similar findings (Gupta et al 1978[14] and Pitkin and Zwirek 1967[7]. It was observed that the level remained constant or increased only slightly up to 34 wks, after which there is a steep rise. After 37 wks, Creatinine level was 2 mg or more in 94% cases.

Doran et al(1970)[4] and Enlander (1972)[15] demonstrated Creatinine level below 1 mg below 28 wks of gestational period and it was 0.66 mg in the present study. In Doran's analysis, the Creatinine level in early gestational period had been shown to have a range of 0.6-1.0 mg. In the present study, the lower level in the one case had been 0.5 mg. Thus a lower level compared to Doran and Enlander had been observed in the early gestational period of 17-23 wks in the present study.

The Uric Acid findings are correlating with that of Doran[16] and Enlander's[15] and are similar to those described by Wolf 1970[17]. The Urea in A.F. increased with the progress of pregnancy, a rise of twice the value in between the two ends of these FIVE groups. The mean concentration at 17-23 wks gestation is 16±0.86 mg and at post maternity zone the range is 29.2 to 32.78 mg in the present study.

Anjan Biswas et al 1986[18] showed that the post matured pregnancy cases contained 32.07±0.36 mg. De et al[19] also showed same range of present study values. The significance P < 0.001 among 36-40 wks gestation & early gestation period was observed. The Urea Nitrogen in the "Pouirst- Scoring System" as proposed by Lind[20] noted the difference between the maternal and A.F. Urea level.

In one study of Creatinine concentration between 20-24 wks had been within a range of 0.5 mg to 0.9 mg. In 24-29 wks gestational period of the present study it had been within the upper limit of their reference range values. Thus, the present study showed a slightly raised pattern when compared to the values of above workers. There has been a greater significance between the maturity zone (16-40 wks) value and that of early gestational period (P<0.001). The present study showed a significance in Creatinine values of (P < 0.01) between the groups 17-23 wks and 24-29 wks. In cases of 30-35 wks of gestation, a mean value of 1.83±0.34 mg was observed. Mandelbaum & Evans [21] described a progressive decrease in A.F. protein with gestation, 1 g / 100 ml of 22 wks declined to 0.5 g / 100 ml at 36 wks. Queenon[22] also suggested same and 300 mg suggests fetal maturity. R. J. Benzie[23] also showed a fall from 500 mg in early weeks to 300 mg as the fetus is matured. The Present study also showed the value in early weeks as 485 mg and falls significantly as the gestation advances. Whitefield 1978[24] showed that the Total Protein content is 4.0 g / litre at 16 wks of gestation and a mean value of 3.0 g/litre at 34-36 wks reflecting a total decrease of 1.0g/litre. The present study is in correlation well agreeing with the findings of other workers. The present study showed the maternal Serum Sodium levels almost constancy at 134 meq/litre and no level of significance, was observed with the rise of gestational age, while the A.F. Sodium showed a negative correlation with the advancement of gestational age. The mean concentration of Sodium was 136meq/litre in early weeks and it decreased to 122-127 meq / litre at term. Thus, the maturity period showing a level of high significance (P< 0.001). The study of Whitefield 1978[24] showed a mean of 136 meq / litre at 16 weeks and 132 meq/litre at 36 weeks.

In the present study, Serum Potassium and Chloride levels remained almost constant at 4.5meq/litre and 100meq/litre respectively. The A.F. Potassium too showed a value of almost 4.4meq/litre with the advancement of pregnancy and no correlation was observed with the rise of gestational wks. But Doran and Benzie[16] showed a slight increase in Potassium levels in A.F. The Chloride values in A.F. showed a constancy of 100 meq / litre, showed no significance. The Study of Hutchinson et al[5] revealed same result, i.e due to entry of HCl from Foetal Stomach to A.F., thus no change in A.F. Chloride values.

CONCLUSION: The Bio-Chemical parameters in A.F. estimated: Creatinine, Uric Acid, Urea, Total Proteins and electrolytes – Sodium, Potassium, Chloride along with Serum electrolytes showed a good correlation with gestational age and foetal maturity. The levels of Creatinine, Uric Acid and Urea in A.F. elevated and Total Proteins decreased with advancing gestation. The Creatinine gave more accurate estimate of Foetal age and maturity than Uric Acid in A.F. but the use of 2 or more parameters along with Urea Nitrogen levels seems to improve the assessment of foetal maturity. The decreasing trend of A.F. Sodium levels with advancing gestation, helped to some extent in assessing foetal maturity. The Serum and A.F. Potassium and Chloride levels remain almost constant throughout the pregnancy. Thus, the use of multiple parameters is desirable for accurate assessment of Fetal maturity.

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Groups	Gestational age in weeks	No. of cases
I(a)	17 – 23	9
I(b)	24 – 29	6
I(c)	30 – 35	6
II	36 – 40	23
III	41 and Above	6
	Total No. of cases:	**50**

Table 1

Weeks of Gestation	**A.F. Creatinine**(in mg/100 ml)			Level of Significance P value
	Range	Mean	SD	
17 – 23	0.48 – 0.84	0.66	±0.09	-
24 – 29	0.67 – 1.47	1.07	±0.20	< 0.01
30 – 35	1.15 – 2.51	1.83	±0.34	< 0.01
36 – 40	2.26 – 3.46	2.86	±0.30	< 0.01
41 and Above	2.90 – 3.53	3.23	±0.15	< 0.01

Table 2(a) : A.F. Creatinine values at different periods of gestation

Weeks of Gestation	A.F. Uric Acid(in mg / 100 ml)			Level of significance P value
	Range	Mean	SD	
17 – 23	1.66 – 3.54	2.60	±0.47	-
24 – 29	3.15 – 4.75	3.95	±0.40	< 0.01
30 – 35	4.60 – 7.20	5.90	±0.65	< 0.01
36 – 40	6.20 – 9.28	7.74	±0.77	< 0.01
41 and Above	10.00 – 11.23	10.15	±0.54	< 0.01

Table 2(b): A.F. Uric Acid values at different periods of gestation

Weeks of Gestation	A.F. Urea(in mg / 100 ml)			Level of significance P value
	Range	Mean	SD	
17 – 23	14.80 – 17.70	16.0	±0.86	-
24 – 29	17.40 – 21.60	19.50	±1.04	P < 0.01
30 – 35	20.00 – 24.94	22.50	±1.22	P < 0.01
36 – 40	23.10 – 27.20	25.13	±1.01	P < 0.01
41 and Above	29.20 – 32.78	31.00	±0.89	P < 0.01

Table 2(c): A.F. Urea values at different periods of gestation

Weeks of Gestation	A.F. Total Protein(in mg / 100 ml)			Level of significance P value
	Range	Mean	SD	
17 – 23	442.46 – 527.54	485	±21.27	-
24 – 29	362.50 – 431.50	397	±17.25	P < 0.01
30 – 35	268.00 – 358.00	313	±22.51	P < 0.01
36 – 40	188.80 – 291.70	240	±25.85	P < 0.01
41 and Above	168.10 – 211.90	190	±10.95	P < 0.01

Table 2(d): Total Protein levels in A.F. in various gestational periods

Weeks of Gestation	Serum(in meq / litre)			A.F.(in meq / litre)			Level of signifi-cance P value
	Sodium	Potassium	Chloride	Sodium	Potassium	Chloride	
17 – 23	134±6.6	4.53±0.92	99.5±5.56	136±2.26	4.34±0.36	101.44±6.72	-
24 – 29	135.33±8.6	4.88±0.8	99.66±5.88	134.66±1.5	4.56±1.04	101.66±6.4	P < 0.01
30 – 35	130.33±6.9	4.35±0.56	103.33±6.52	130.0±1.63	4.25±0.42	102.0±7.14	P < 0.01
36 – 40	135.13±8.76	4.5±0.96	101.26±7.66	126.52±2.19	4.47±0.78	101.2±7.62	P < 0.01
41 and Above	134.33±6.76	4.66±1.44	101.16±5.98	126.0±1.15	4.38±0.70	100.0±7.58	P < 0.01

Table 2(e): Values of Sodium, Potassium, Chlorides in A.F. and Serum in various gestational periods

A.F. Parameter	Correlation Co-Efficient
Creatinine	r = 0.82
Uric Acid	r = 0.93
Urea	r = 0.96
Total Protein	r = - 0.98
Sodium	r = - 0.95

Table 3: Correlation pattern of the parameters with Advancing gestation

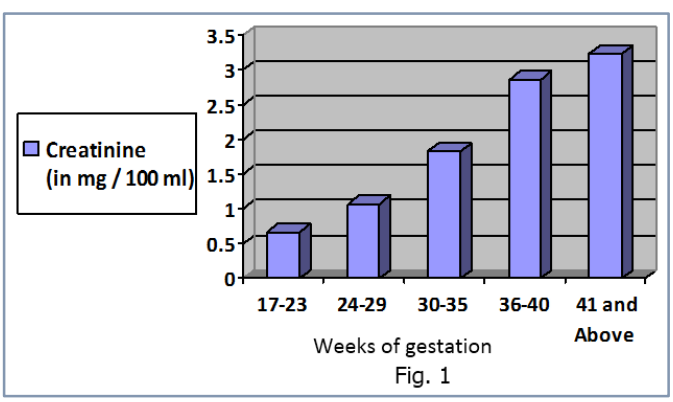

Fig. 1

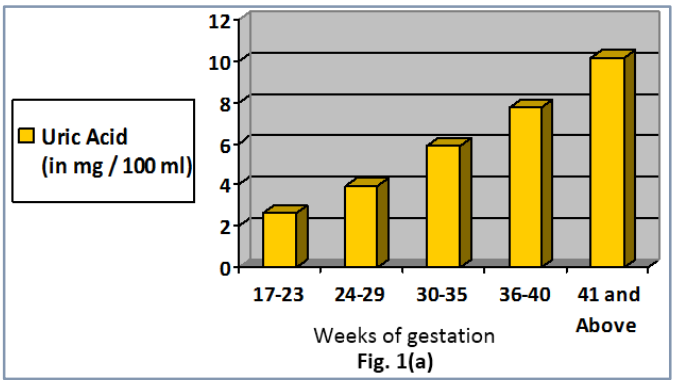

Fig. 1(a)

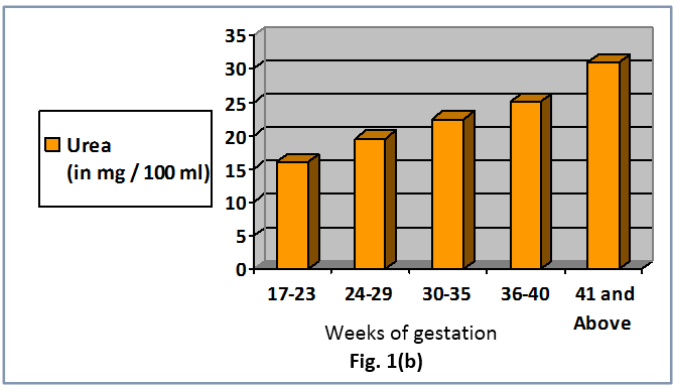

Fig. 1(b)

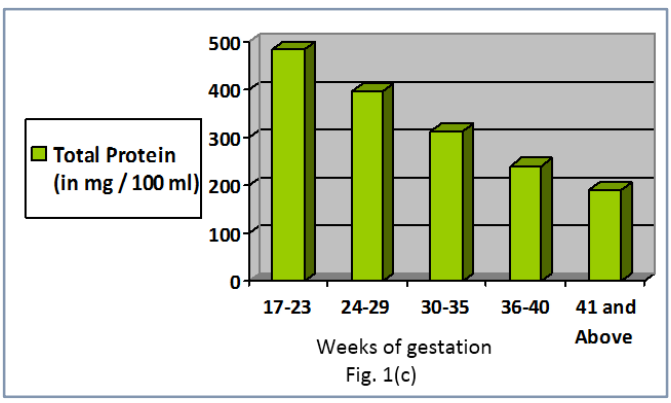

Fig. 1(c)